Edited by D. Rice August 7, 2000

DR. RICE: I also want to thank the organizers for inviting me here today. I was a little bit perplexed about what to talk about today, since neurotoxicity risk assessment is such a broad issue. What I decided to do is to use lead and PCBs as exemplars to make a series of points, and I hope that this doesn't end up being too disjointed.

Comparison of Effects Lead -- Human

- Children 1-10 μg/dl
  - IQ deficits
  - impaired school performance
  - distractibility, short attention span
  - impulsivity
  - perseveration
  - increased activity

What I would like to do first is present a comparison between effects in adults and effects in developing organisms for both lead and PCBs. If we look at the effects of lead in humans, lead produces, in industrially exposed individuals, peripheral neuropathy and sensorimotor deficits. That lead produces these effects is very clear. Researchers have also reported deficits on cognitive tests, although it is unclear whether these are really sensorimotor rather than cognitive effects. These effects in adults occur at blood lead levels of about 30 to 40 micrograms per deciliter. If we look at children, there is a range of effects that have been identified: I.Q. deficits, impaired school performance,

distractibility and short attention span, impulsivity, perseveration in nonadaptive behaviors, as well as increased activity. I'm going to talk about some of these effects as the talk goes on.

Comparison of Effects Lead -- Human

- Children 1-10 μg/dl
  - IQ deficits
  - impaired school performance
  - distractibility, short attention span
  - impulsivity
  - perseveration
  - increased activity

With respect to the body burden at which these effects occur, I have indicated one to ten microgram per deciliter on the slide. Of course, the CDC level for action is 10 micrograms per deciliter, but I think there is really pretty good evidence now that effects certainly occur below 10 μg/dl. For example, the meta-analysis that Joel Schwartz did a number of years ago failed to find a threshold down to one microgram per deciliter.

Comparison of Effects Lead -- Animal

- Adult
  - locomotor effects (weight loss)
  - cognitive studies largely negative

In adult animals, lead produces changes in motor activity, often in the presence of overt toxicity such as weight loss. Cognitive studies have been largely negative in the rodent, and it is certainly the case that if all we knew about lead was based on adult rodent testing, lead would never be regulated on the basis of neurotoxicity. Lead would probably be regulated on the basis of toxicity in other organ systems, such as nephrotoxicity, based on the adult rat.

Comparison of Effects Lead -- Animal

- Developmental (10μg/dl monkey, <20 μg/dl rat)
  - learning deficits
  - distractibility
  - short attention span
  - impulsivity
  - perseveration
  - increased activity

If we go on to look at developing animals, including rats, effects are very similar to the effects in humans: learning deficits, distractibility, short attention span, impulsivity, perseveration and increased activity. All these effects can be measured in animal models, and I'm going to talk about that a little later.

In terms of the body burden at which effects are observed, in our laboratory we worked with a number of cohorts of monkeys. The lowest dose resulted in blood lead levels of 10 micrograms per deciliter, compared to “control” monkeys with blood levels of two or three μg/dl. It is important to remember with respect to lead that there is no “control group” in any population. All of us are exposed at two to three orders of magnitude above what background levels were before anthropogenic exposure began. In our experiments, we found effects in monkeys with blood levels of 10 compared to those with 3 μg/dl. In work done by Deborah Cory-Slechta in the rat behavioral deficits were identified at 20 μg/dl blood lead, the lowest level that she studied. This was a series of studies in which exposure began postweaning, so was not comparable to any US EPA guidelines. There is apparently very good congruence between humans and animal

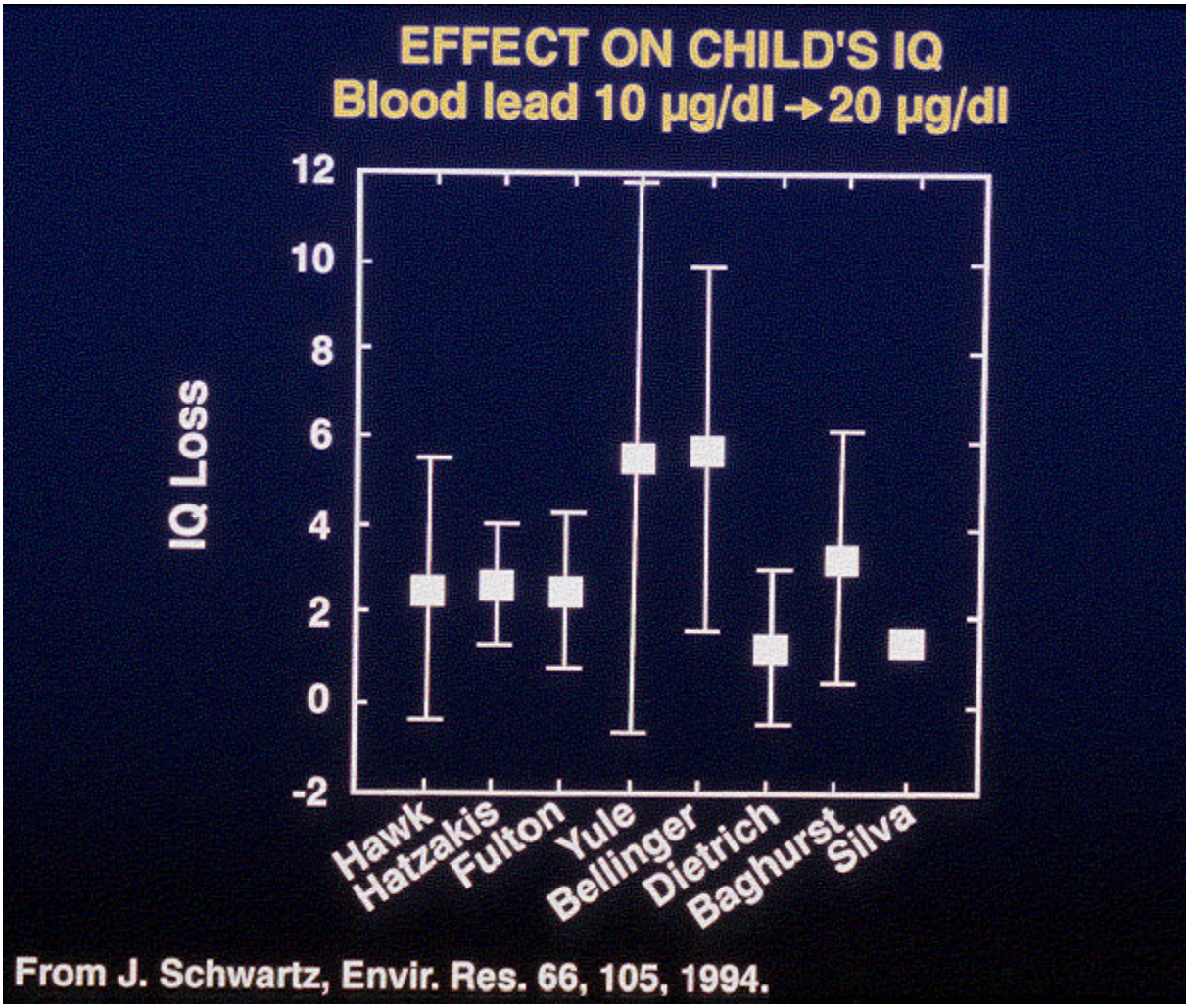
models in terms of the body burden at which effects are found. That's very different from derived RfDs, which I will discuss at the very end.

If we do the same kind of comparisons for PCBs, what are identified in adults are paresthesias in people who were very highly exposed in environmental tragedies.

Comparison of Effects PCBs -- Human

- Adult
  - paresthesias
- Child 1-3 ppb
  - IQ deficits
  - impaired school performance
  - impulsivity

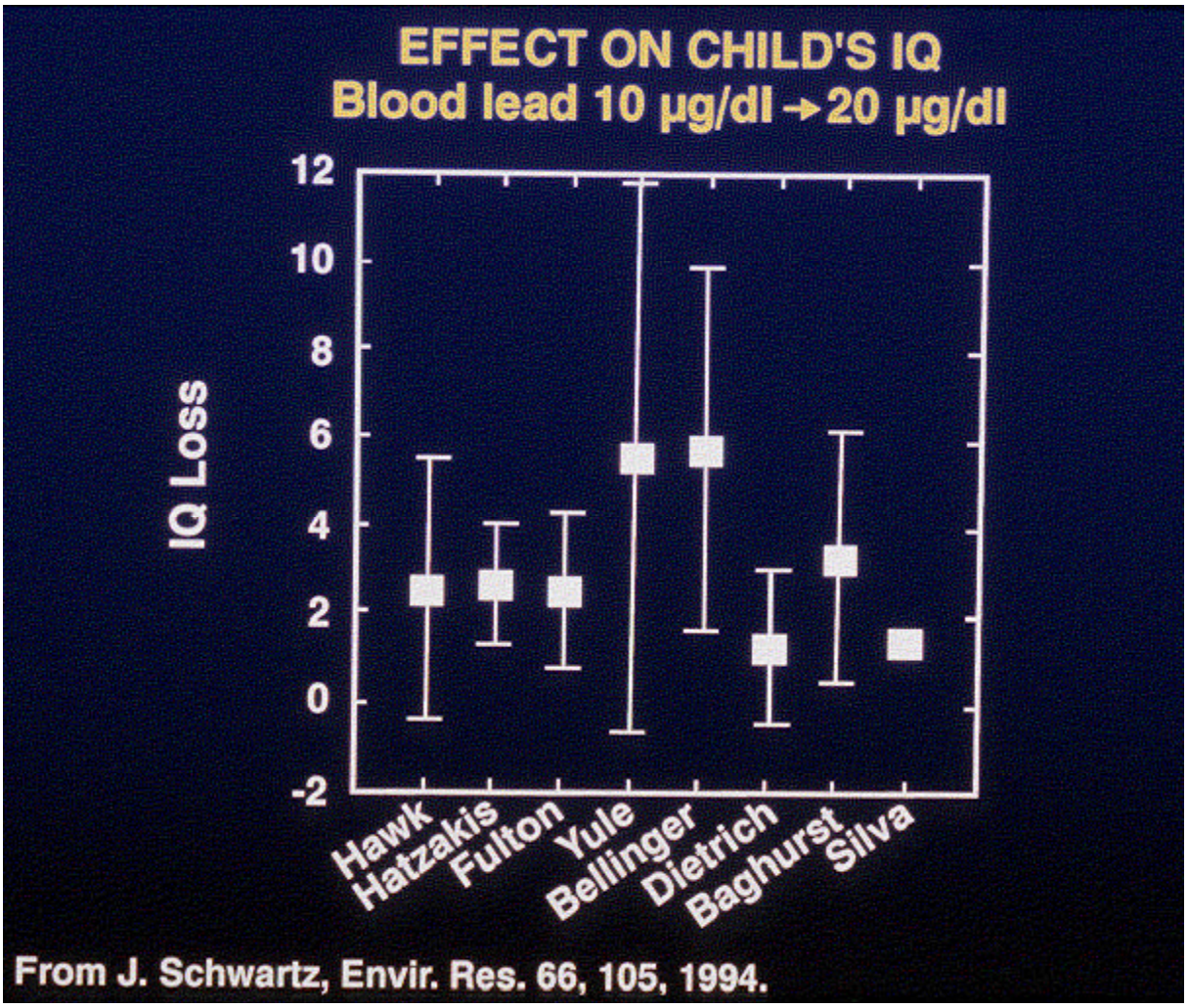
In children, we see effects on I.Q., impaired school performance, impulsivity, and impairment of attention and memory. The behavioral processes affected by PCBs have not been as well explored as they have for lead. These effects occur at one to three ppb in blood. This is not a threshold for effects; these effects are occurring at environmental levels that you and I and everybody in the room are carrying right now. In adult animals, PCBs produce locomotor effects in the presence of fairly profound weight loss. Similar to lead, based on adult rats, PCBs would never be regulated as a neurotoxicant.



For developmental exposure to PCBs, learning deficits have been identified in animals, as well as perseverative behavior, impulsivity, and deficits in memory. In a study in our laboratory in which monkeys were exposed postnatally only to PCBs, we found effects at 1.5 to 3 ppb in the monkey. This is not a threshold; this is the only dose we had. This was a congener mixture that was representative of the congeners found in breast milk. The monkey is born more mature than the human, as opposed to the rat which, of course, is born less mature. Postnatal exposure in a rat may be in that prenatal human window; in the monkey that's not true, so any exposure would actually be comparable to beginning dosing a human at probably several months of age.

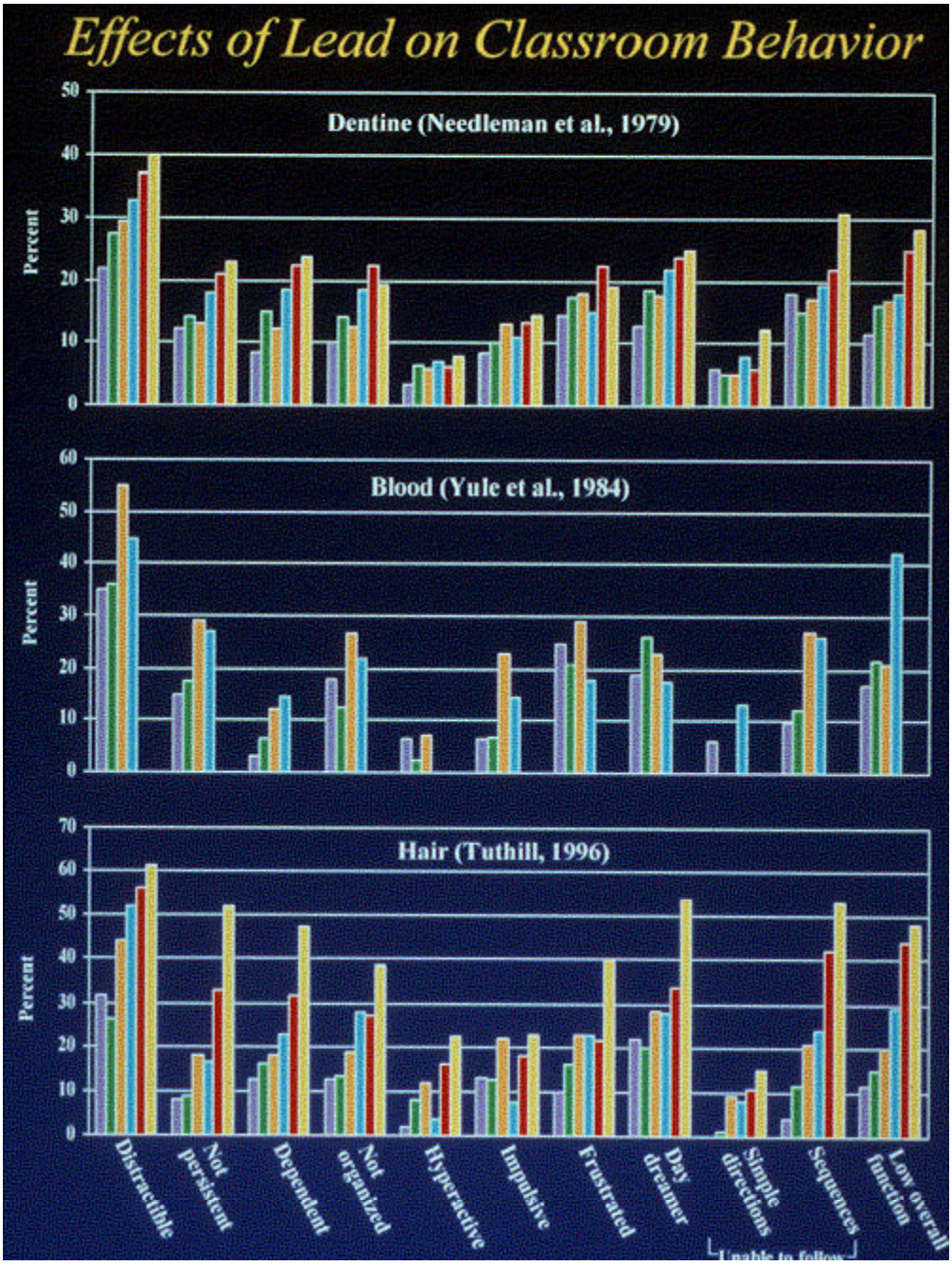
For PCBs, as for lead, there is good congruence between the human and the animal data in terms of the body burden at which effects occur. There is also very

good congruence with respect to the kinds of behavioral deficits that are identified following developmental exposure.



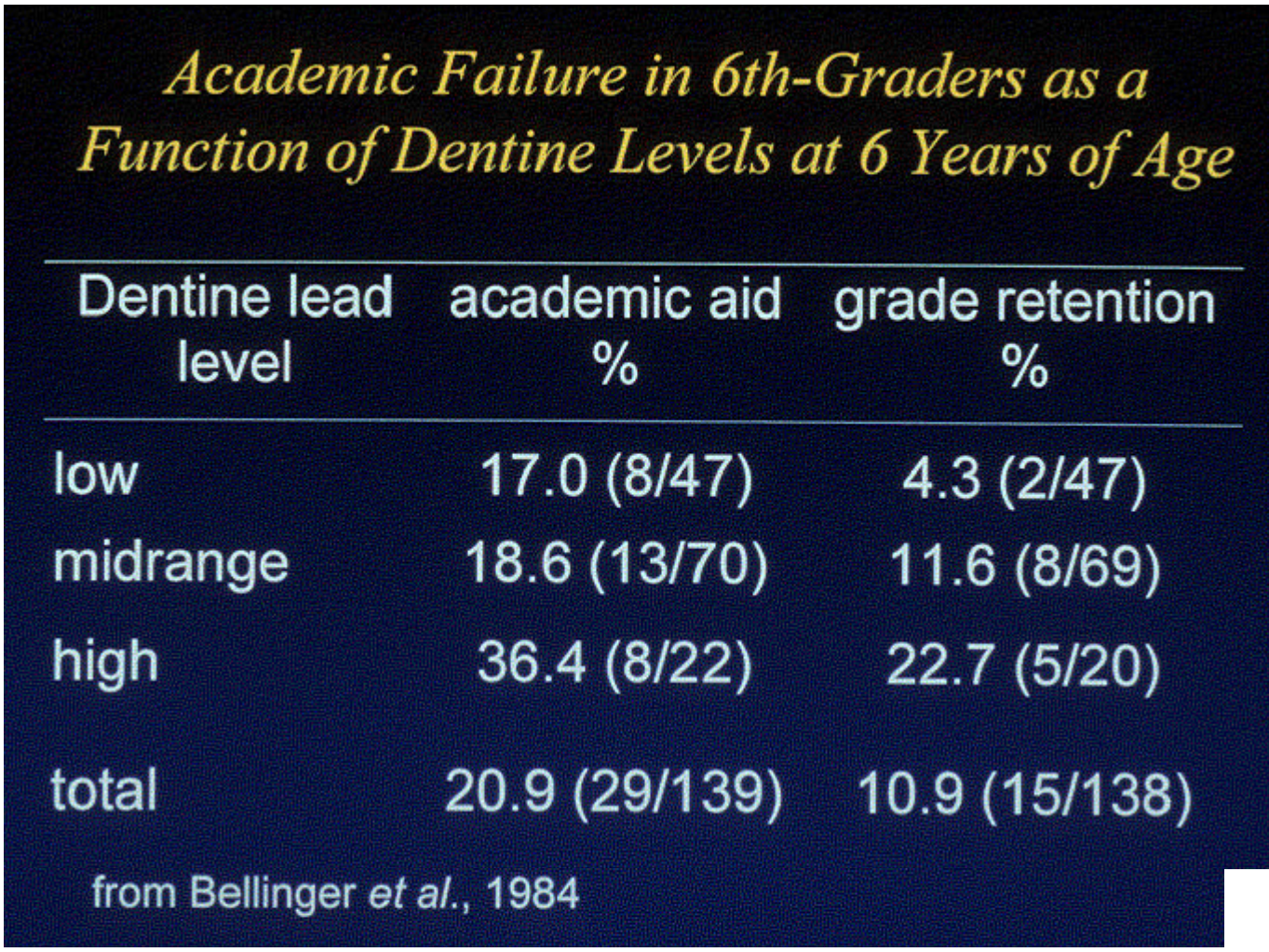
Schwartz, J. Environ Res. 66, 105-124, 1994

Next I'm going to present a series of specific effects of lead or PCBs in children and animal models, in order to make a number of points. Here is a figure from a paper by Joel Schwartz, 1994, in which he looked at the I.Q. loss over a series of studies. There are more studies available now, and they are presented in the WHO lead document. I.Q. decrements vary between two and six I.Q. points, as the child's blood level increases from 10 to 20 micrograms per deciliter. The next slide presents effects of lead on classroom behavior as assessed by a teacher's rating scale.

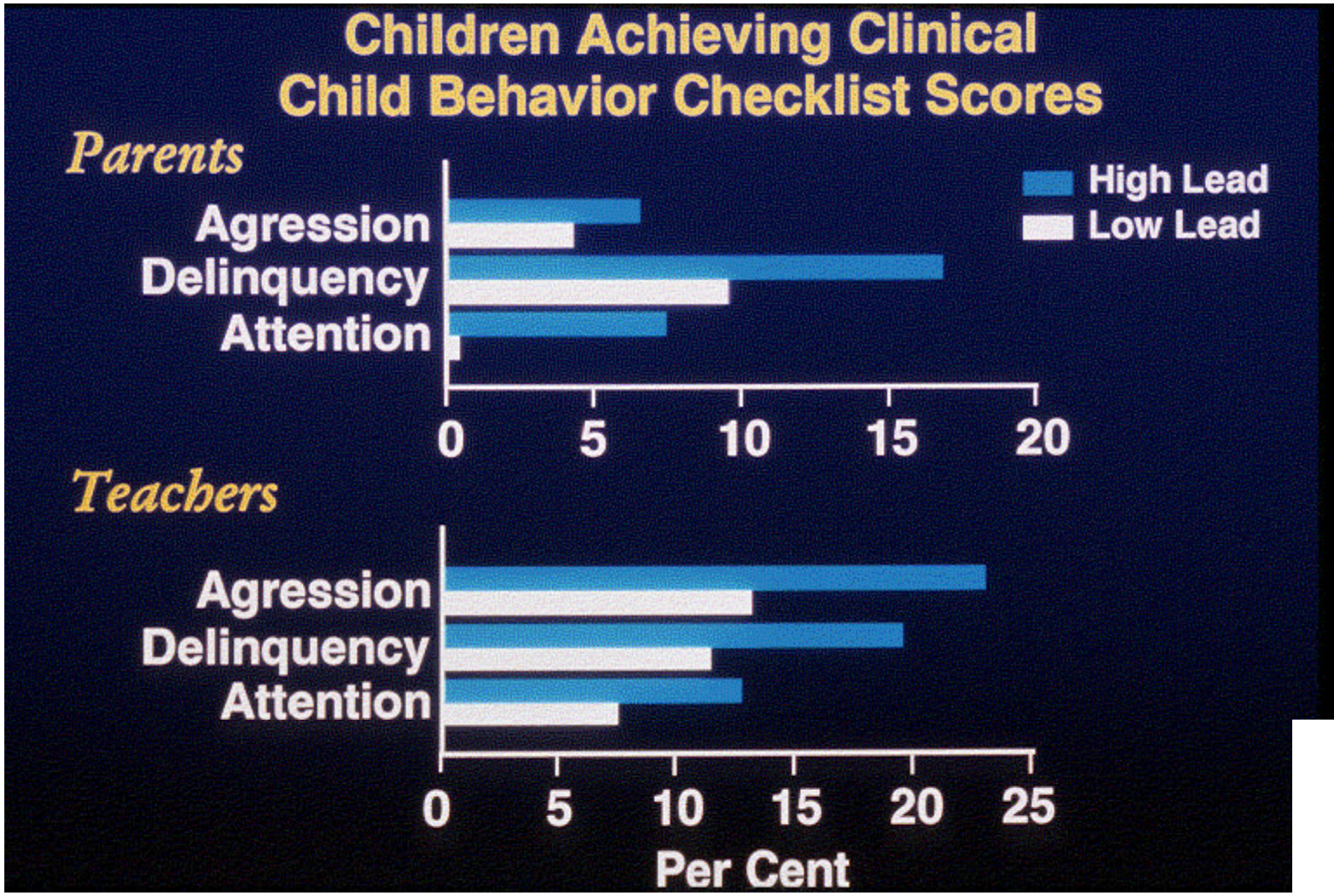


The top panel is the initial Needleman 1979 study. The middle panel is a subsequent study published in 1984 by Yule et al. And the bottom panel depicts a more recent study, 1996, using hair as the biomarker of lead exposure. The categories rated by the teachers included distractibility, not persistent, dependent, not organized, hyperactive, impulsive, frustrated, a daydreamer, and unable to follow simple and complex sequences

of directions. The positive rating increases as a function of body burden of lead, with very good congruence amongst these studies.



The next slide depicts requirement for academic aid related to lead exposure. This Bellinger et al. study assessed the 1979 Needleman cohort. The percentage of academic aid, requirement for academic aid and grade retention in sixth graders increases as a function of dentine lead at six years of age.



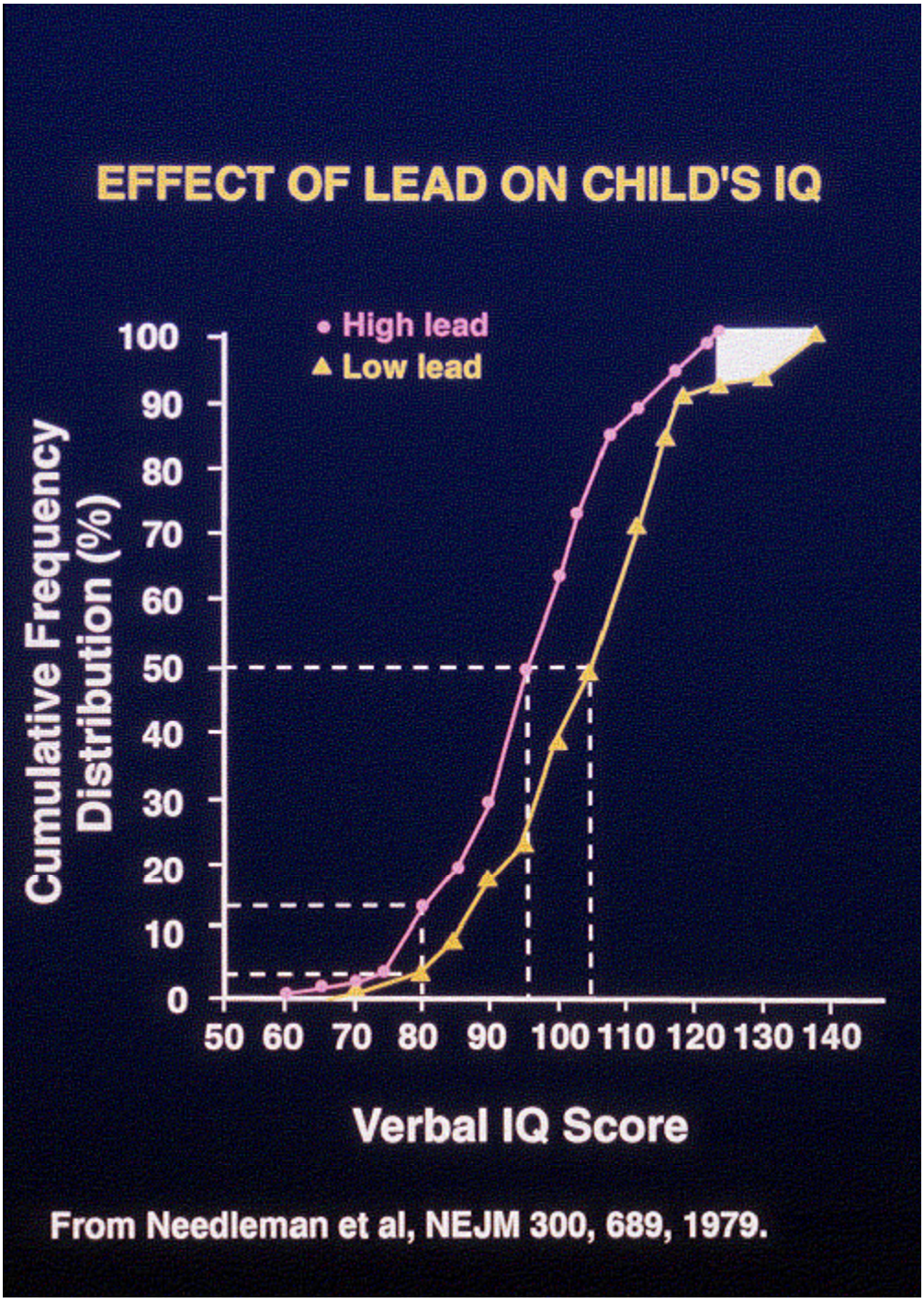
This shows the proportion of children achieving a clinical Child Behavior Check List score. In other words, these are children who would be identified by clinicians as having behavioral problems. These results are based on both a parent's scale questionnaire and a teacher's scale. Needleman et al. have also studied a different cohort of children. Needleman et al. looked at aggression, delinquency, and attentional deficits. They observed an increase in this kind of problem behavior as a function of lead level as identified by both parents and teachers. In fact, these kids self-report more delinquent behaviors as 11-year-olds than their compatriots with lower lead levels. As kids become older deficits such as impulsivity, distractibility, and failure to persist begin to have important and perhaps long-term consequences.

We are increasingly aware that *in utero* exposure to cigarettes, or maternal alcohol consumption, results in increased antisocial or criminal behavior 30 years later. There is evidence for these kinds of effects for lead as well. The Justice Department is now interested in lead exposure for these very reasons.

A *small* effect is not necessarily
an *unimportant* effect.

Needleman H.L. et al., JAMA 275- 363-369, 1996

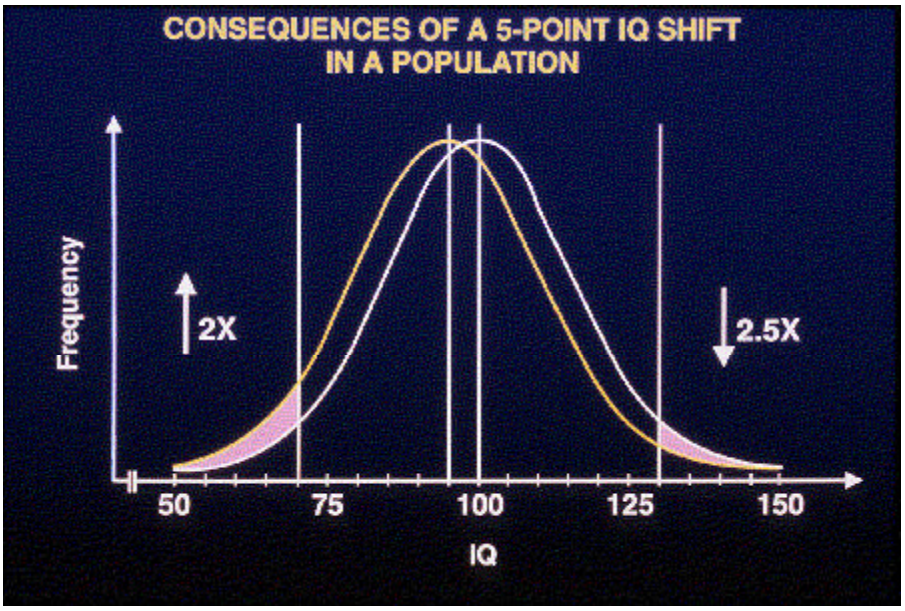
It is important to understand that a small effect is not necessarily an unimportant effect. I'm going to talk about that in a couple of ways.



Needleman H. et al., NEJM 306, 307, 1982

This slide is from Needleman's 1979 study, depicting cumulative I.Q. There was an average of six points difference on the mean between the high-lead and low-lead children. What he found was that there were no kids in the high-lead group with I.Q.s above 130. Similarly, there was an increased frequency of kids with lower I.Q.s,

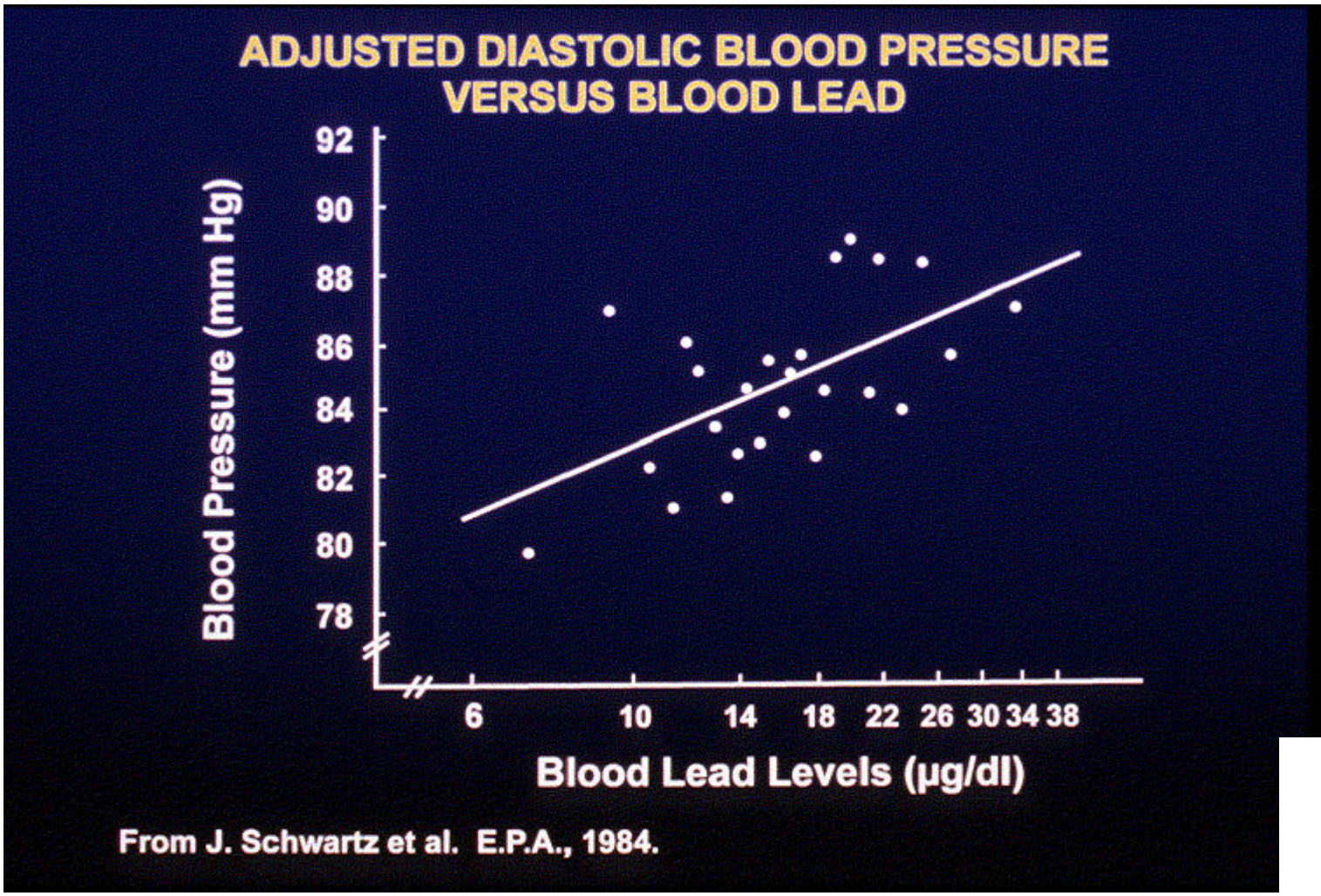
including those that would be considered, or identified clinically, as mentally retarded. A relatively small shift in the mean has dramatic effects on the tails of the distribution.



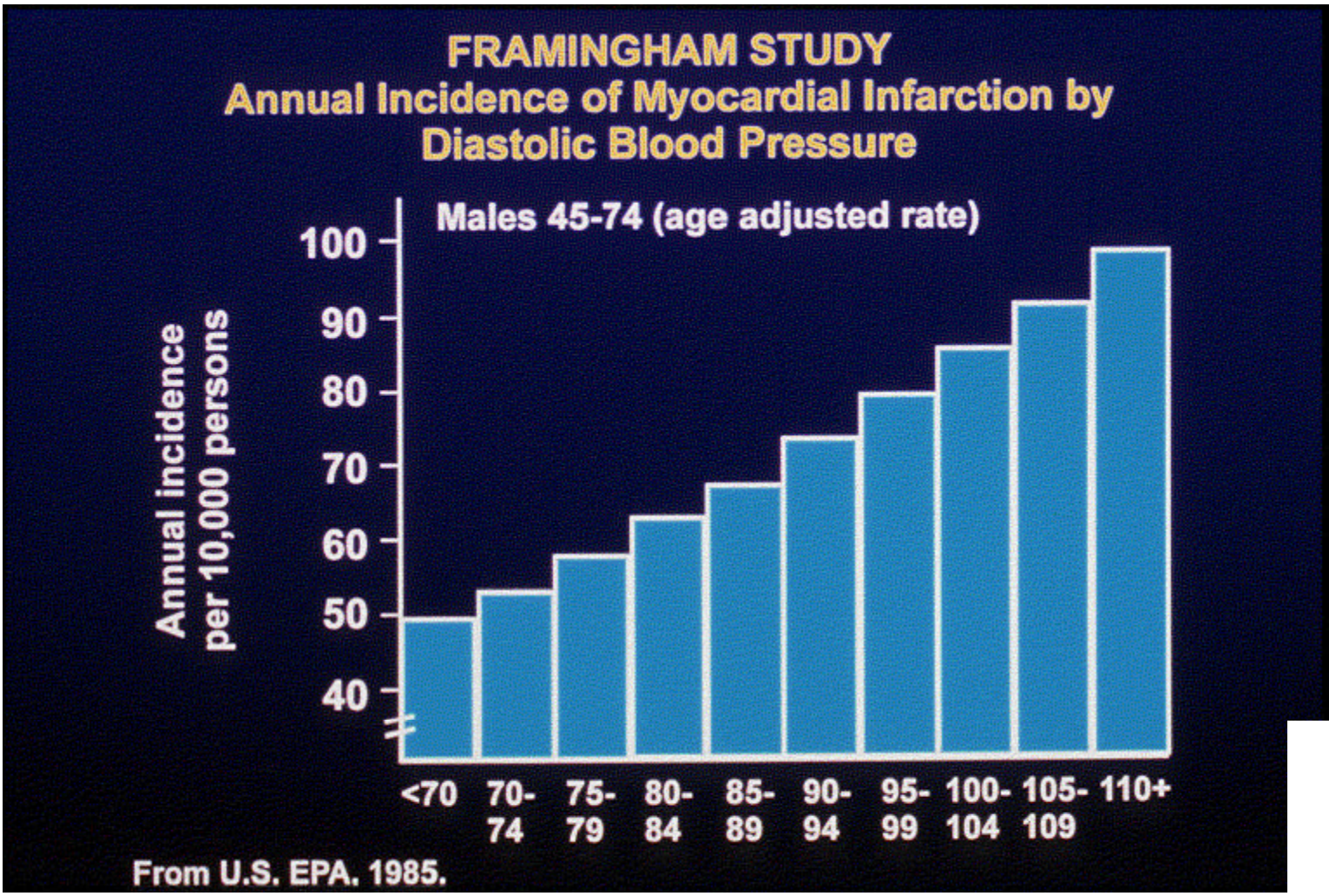
The next slide demonstrates that more explicitly. This slide depicts consequences of a five point I.Q. shift in the population, assuming that the distribution remains normal. The result is a two-and-a-half times decrement in kids with I.Q.s above 130, and a doubling of kids with I.Q.s less than 70. Such effects, if they're population-wide, have really serious consequences for the population.

Estimated Savings for 1 µg/dl reduction in blood lead (from J. Schwartz, 1994)

Children	*Millions/year 1994 $ US*
Medical costs	189
Compensatory education	481
Earnings	5,060
Infant mortality	1,140
Neonatal care	67
Total	**6,937**

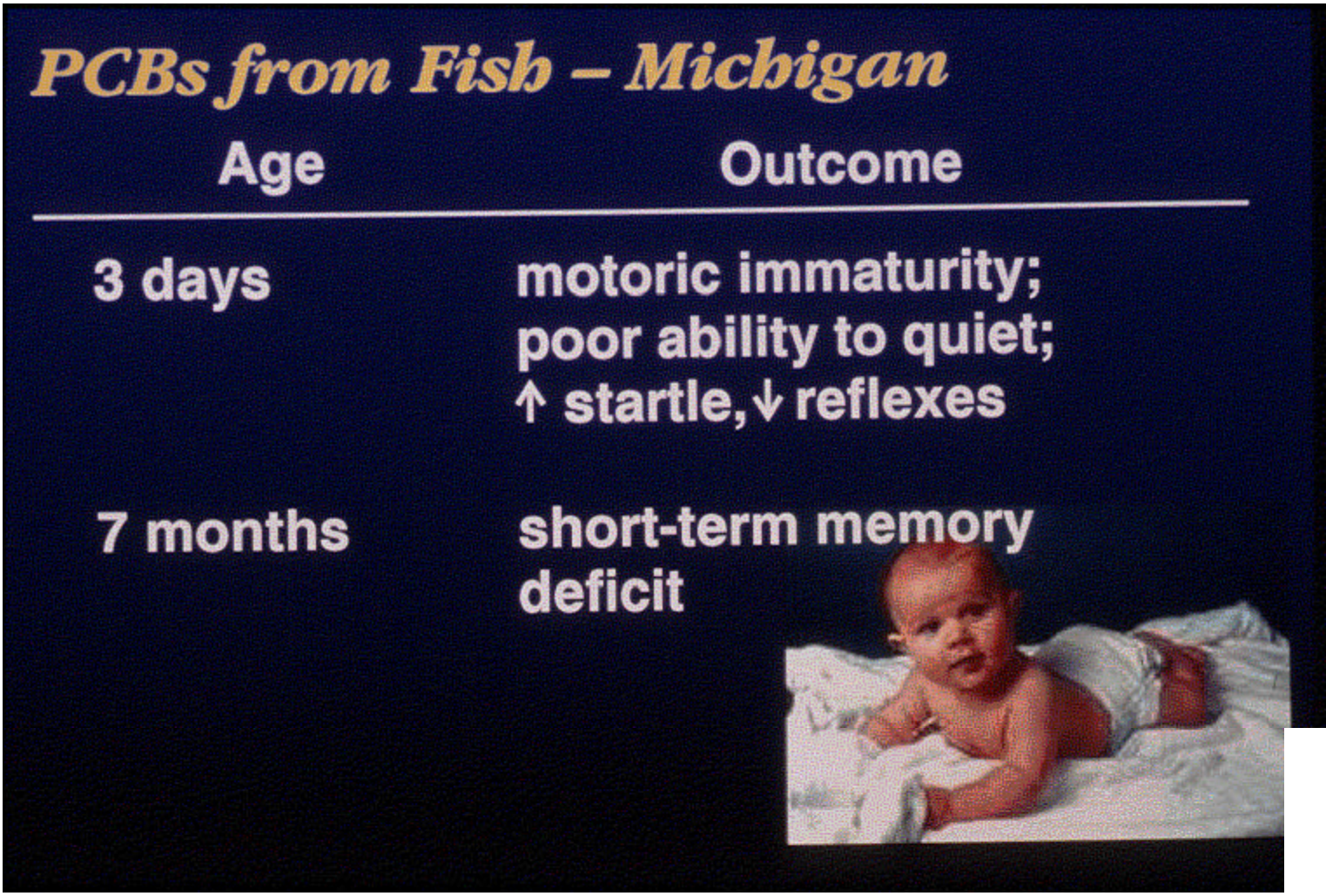


This shows the relationship between blood pressure and blood lead levels. Now, this is not children, but I'm presenting these data to make a point. Diastolic blood pressure increases as blood level goes up beginning between six and 10 micrograms per deciliter: relatively modest increases in blood lead levels. Similarly, the diastolic blood pressure is not really high. These people would probably be told to "get some exercise and watch your diet." The next slide shows the relationship between diastolic blood pressure and myocardial infarction. At diastolic blood pressures that would not be considered clinically significant there is already an increase in the incidence of myocardial infarction.



The next slide (Slide 29) depicts an increase in death associated with diastolic blood pressures that, again, are not necessarily in a range that your physician would be worried about. In fact, there has been a recent paper directly linking blood lead levels to increases in myocardial infarctions.

The reason for dwelling on this issue is that there is often confusion regarding the importance of small changes in animal studies. There are a couple of points that need to be kept in mind. First, based on the small number of animals in a typical study, it is impossible to know what the distribution would be in large population of rats, for example, assuming or course that the rat is a good model for the endpoint under investigation. Second, even very small changes that don't result in clinical illness in an individual can nonetheless be important, if they're spread over an entire population. When we're analyzing our studies and we see a little increase in glucose levels, or a little decease in hematocrit, don't dismiss those as not being meaningful because they do not result in illness. Think about the effects in the human population of a small change in IQ or blood pressure.

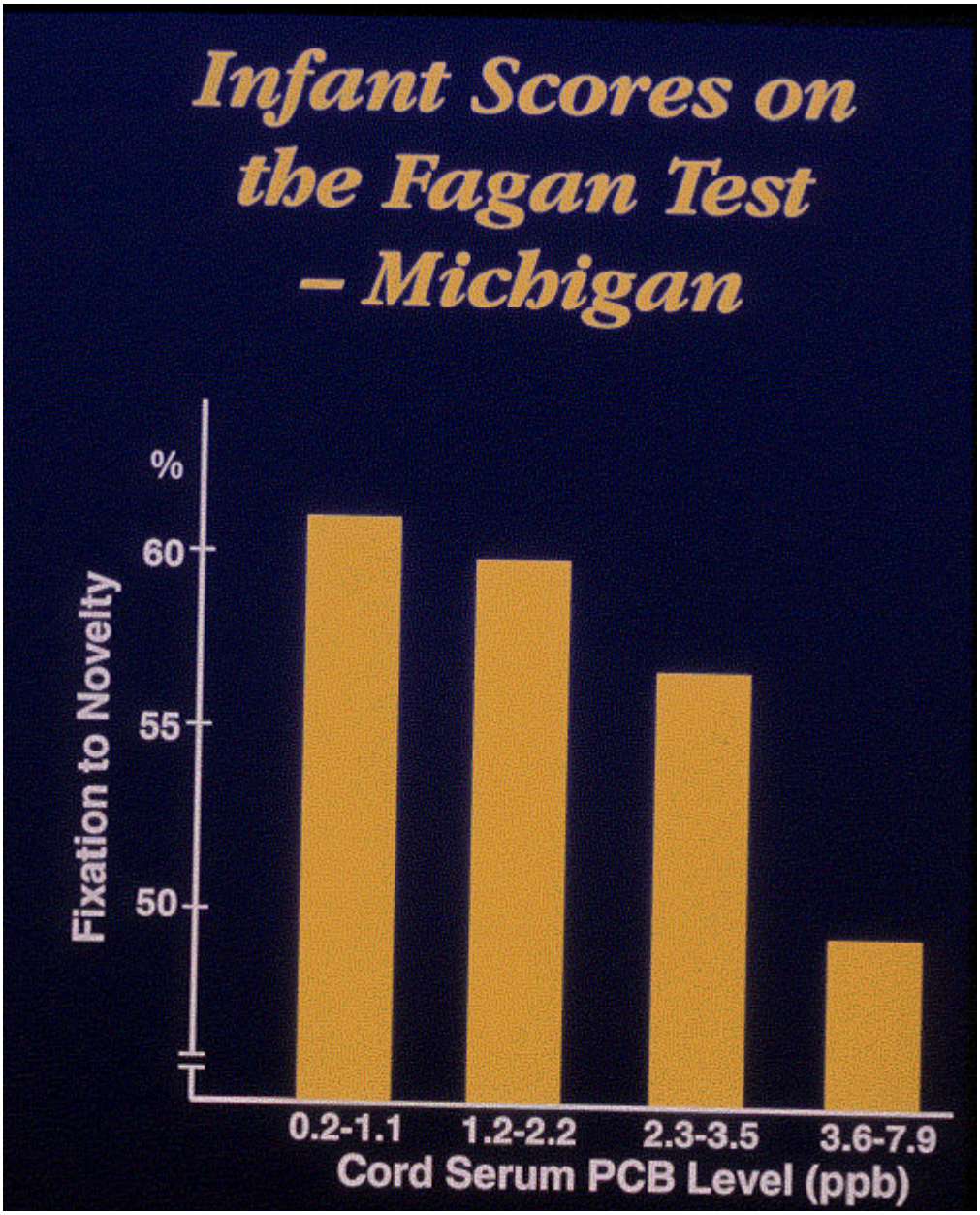


Now I'm going to switch gears and talk about the effects of *in utero* exposure to PCBs. This slide is from the Michigan study, which studied the offspring of women who consumed Lake Michigan fish. During infancy these babies exhibited motoric immaturity, poor ability to quiet, increased startle response, and decreased reflexes. A short-term memory deficit was identified in these infants at seven months of age.

PCBs from Fish -- Michigan

- Fagan test of infant memory
- Predictive of later IQ
- Cord serum PCB greater than 3 ppb 3X more likely to score in the lower tail of the distribution

The next slide is the Fagan test of infant memory, which is a relatively good predictor of later I.Q., unlike some of the tests typically used in young children. For those of you who don't know what this test is, a baby is facing a picture, typically a person’s face. After a several second delay, the child is presented with two pictures, one the picture she's already seen, and the other a new face that she hasn't seen before. Babies, like the rest of us, prefer novelty to the familiar, and so a normal baby, if she remembers seeing the first picture, will look longer at the novel picture. This is a measure of at least short-term memory, and attentional processes as well.



Jacobson S.W. et al., J. Child Dev. 56, 853-860, 1985

The Jacobsons found that infants with cord serum PCBs greater than three ppb were three times more likely to score in the lower tail of the distributionIn fact, the highest-exposed kids have a fixation to novelty of 50%. In other words, they apparently don't remember.

PCBs from Fish -- Michigan

Age	Outcome
11 years	↓full-scale and verbal IQ, ↓word and reading comprehension, ↓memory and attention

Here is data from the same study when the children were 11 years old. A decrease in full-scale and verbal I.Q., a decrease in word and reading comprehension, and a decrease in memory and attention were associated with various measures of *in utero* PCB exposure. The highest PCB group had an I.Q. of six points lower than the average.

PCBs from Fish -- Michigan

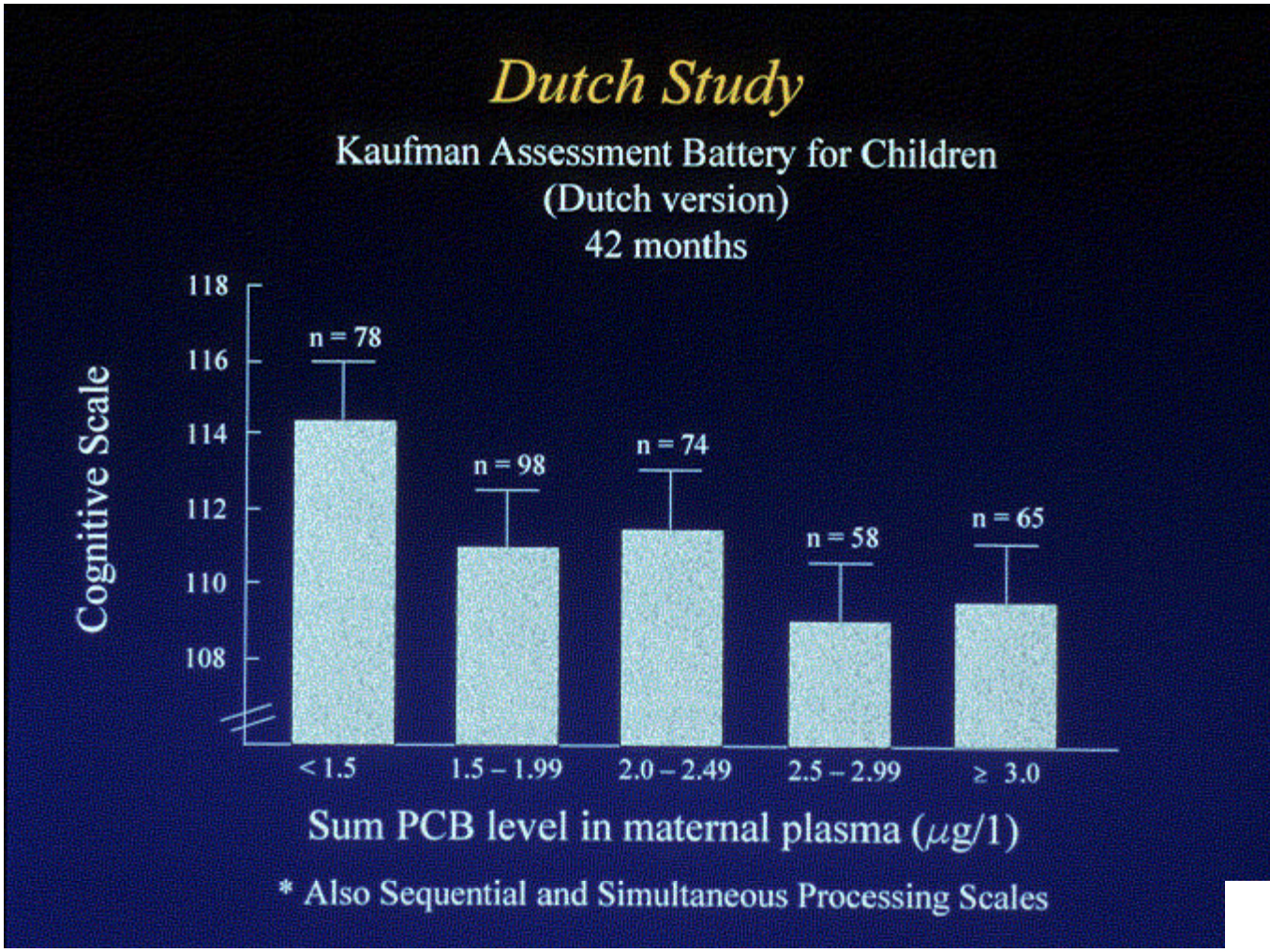
- Highest PCB group had IQ 6 points lower than average.
- 3X more likely to have low Iqs
- 2X more likely to be at least 2 years behind in reading ability

This group was three times more likely to have low I.Q.s, and twice as likely to be at least two years behind in reading ability. What do you think the consequences of that are going to be in five years?

PCBs from Food -- The Netherlands

Age	Outcome
10 and 21 days	Decrease in reflexes (breast milk) Hypotonicity (breast milk)
3 months	Lower psychomotor scores (prenatal)
7 months	Lower psychomotor scores (breast milk)

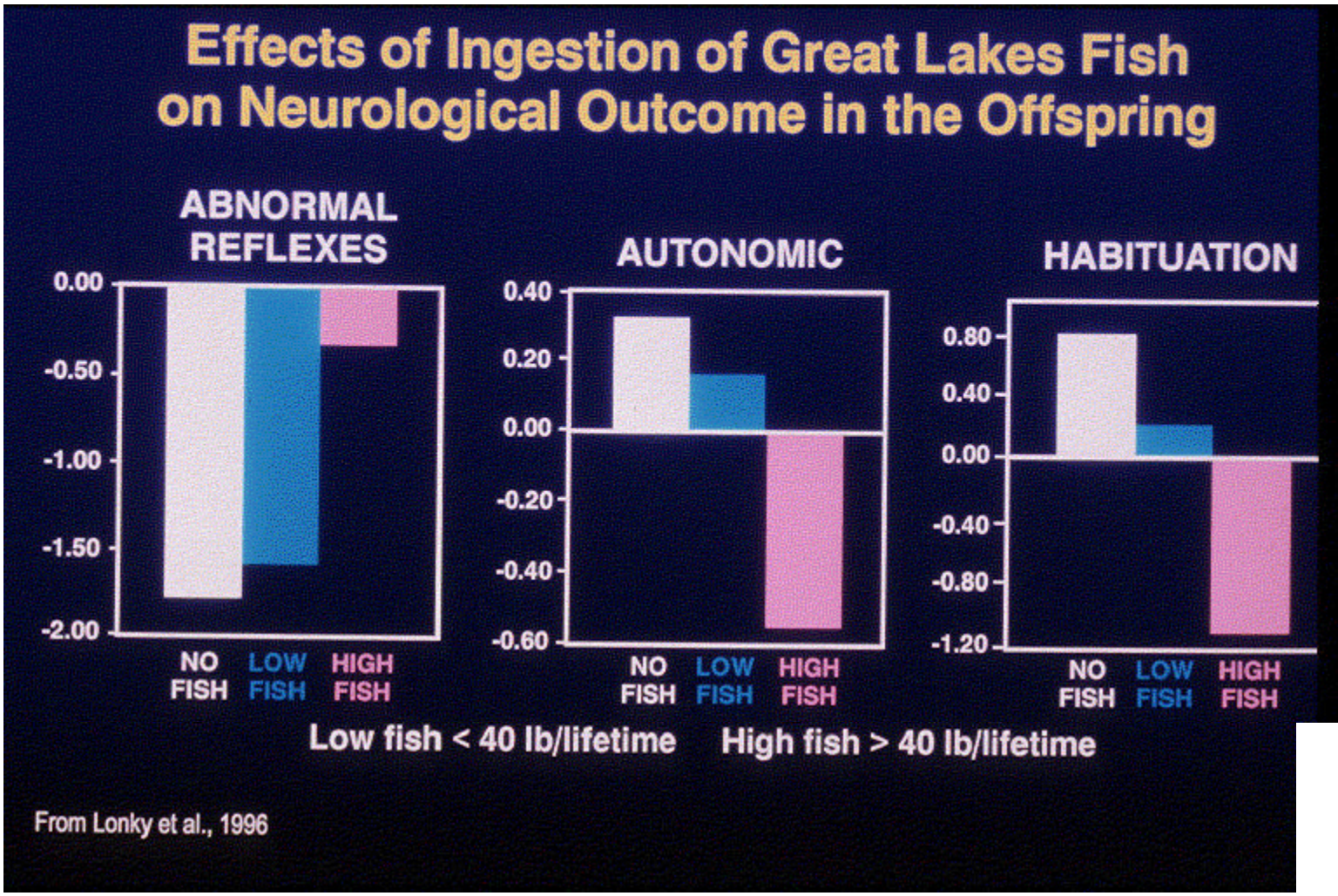
This is from a study from the Netherlands, which is ongoing. Exposure in this study to PCBs is from food. This is not a fish-eating population; most of the PCB exposure is through dairy products and processed food. The investigators of this study found the same effects that the Jacobsons found early on in terms of decreases in reflexes. They also found, at seven months, a lower psychomotor score that was associated with exposure through breast milk, not prenatal exposure.



Patandin S. et al., J. Pediatr. 134, 33-41, 1999

The next slide shows the cognitive effects of PCB exposure at 42 months of age in the children. As PCB levels in maternal plasma increase from less than 1.5 to more than three ppb in blood, there is a dose-dependent decrease in I.Q. It is important to emphasize that these are PCB levels that are typical levels observed in the general population. In addition to these results, which were associated with *in utero* exposure to PCBs, this study has also identified deficits on a vigilance task, which measures attention, associated with PCB levels, as well as deficits in complex play behavior associated with concurrent exposure.

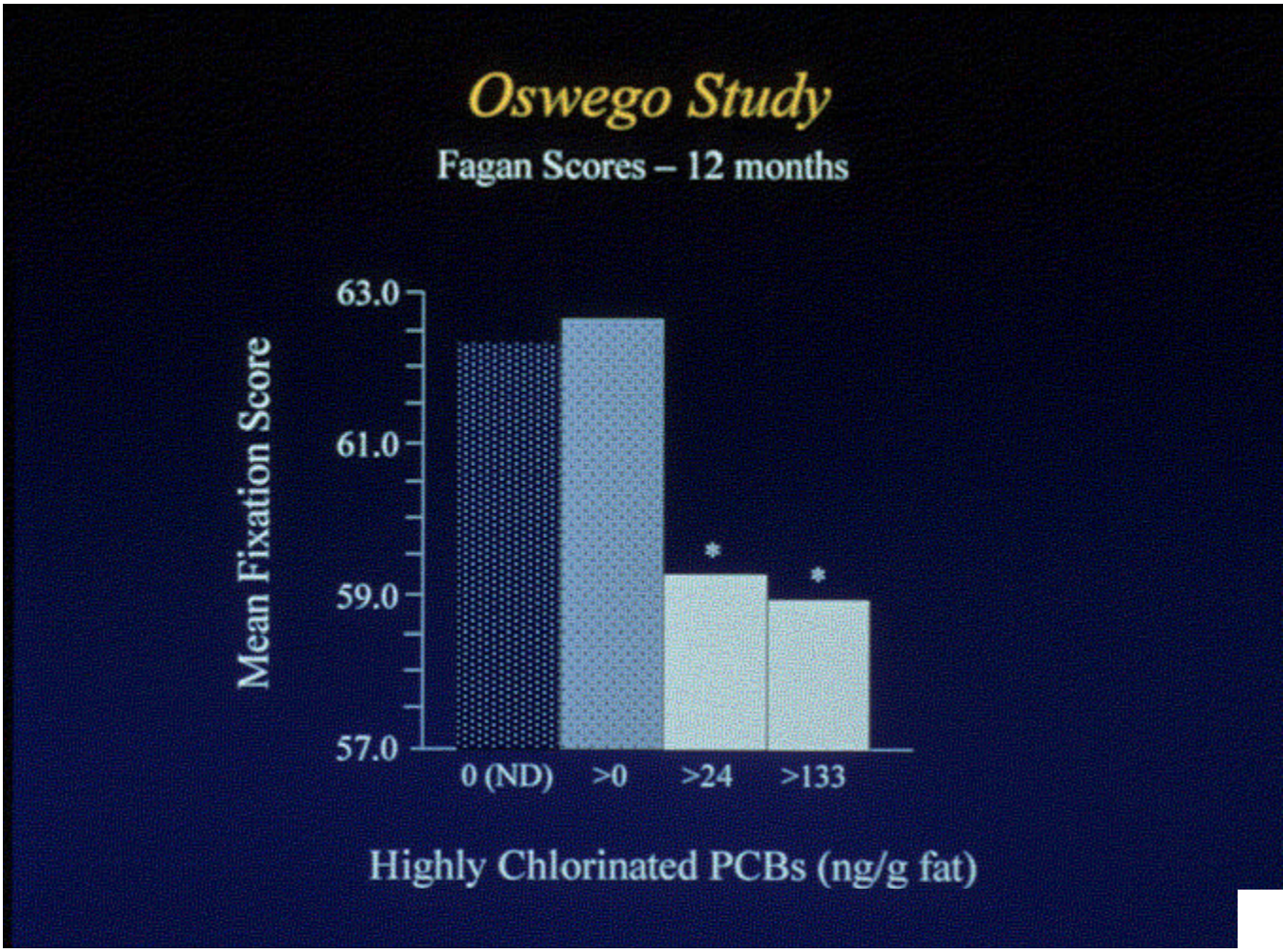
I don't think that we know, as has been suggested, that PCBs only produce effects as a result of prenatal exposure. Biologically it doesn't make sense that that would be true. Development isn't over at birth. The kinds of processes that are affected by PCBs continue after birth, so it really only makes sense that effects probably are going to continue to be seen as these populations age, associated with concurrent, as well as prenatal exposure.



Lonky E. et al., J. Great Lakes Res. 22, 198-212, 1996

The last study I would like to describe is another Great Lakes fish-eating study in Oswego, New York. PCB levels in Great Lakes fish have decreased since the Jacobson study, but the results of this study are largely replicating the Jacobson study,

only at lower exposure. The initial paper reported an association between adverse effects during infancy with fish intake. High fish intake was defined as greater than 40 pounds of fish in the lifetime of the mother, weighted for kind of fish and age of fish to account for the fact that PCB levels are higher in bigger fish in certain species of fish. The results in general replicate the findings of the Jacobson study, identifying abnormal reflexes, autonomic function, and habituation. These effects are also associated with PCB levels in mothers' blood.



Darvill T. et al., Neurotoxicol. in press

The next slide is the results of the Fagan test from the Oswego study. Basically the results are the same as those in the Jacobson study: mean fixation time decreases as cord blood level increases.

EPA Test Battery -- Adult Rats

- Functional and Observational Battery
  - forelimb and hindlimb grip
  - landing foot splay
  - gross sensorimotor reactivity
- Motor Activity

I'm going to switch to talking about methodology for looking at behavior in animals. The US EPA test battery for adult rats includes a functional observational battery, fore- and high-limb grip strength, landing foot splay, gross sensorimotor activity, and pain response, in addition to motor activity. This battery is designed as a screening battery to identify neurotoxicity.

EPA Test Battery -- Developing Rats

- Motor Activity
  - ontogeny
- Auditory Startle and Habituation
  - weaning and 60 days
- Learning/Memory
  - weaning and 60 days

The next slide represents the tests designed for the developing organism. This battery includes the ontogeny of motor activity. Auditory startle and habituation are performed at weaning and at 60 days, as are tests of learning and memory. When you ask the question, "Is the developing rat more sensitive than the adult rat," it is important to recognize that there is very little congruence between the adult and developing test batteries.

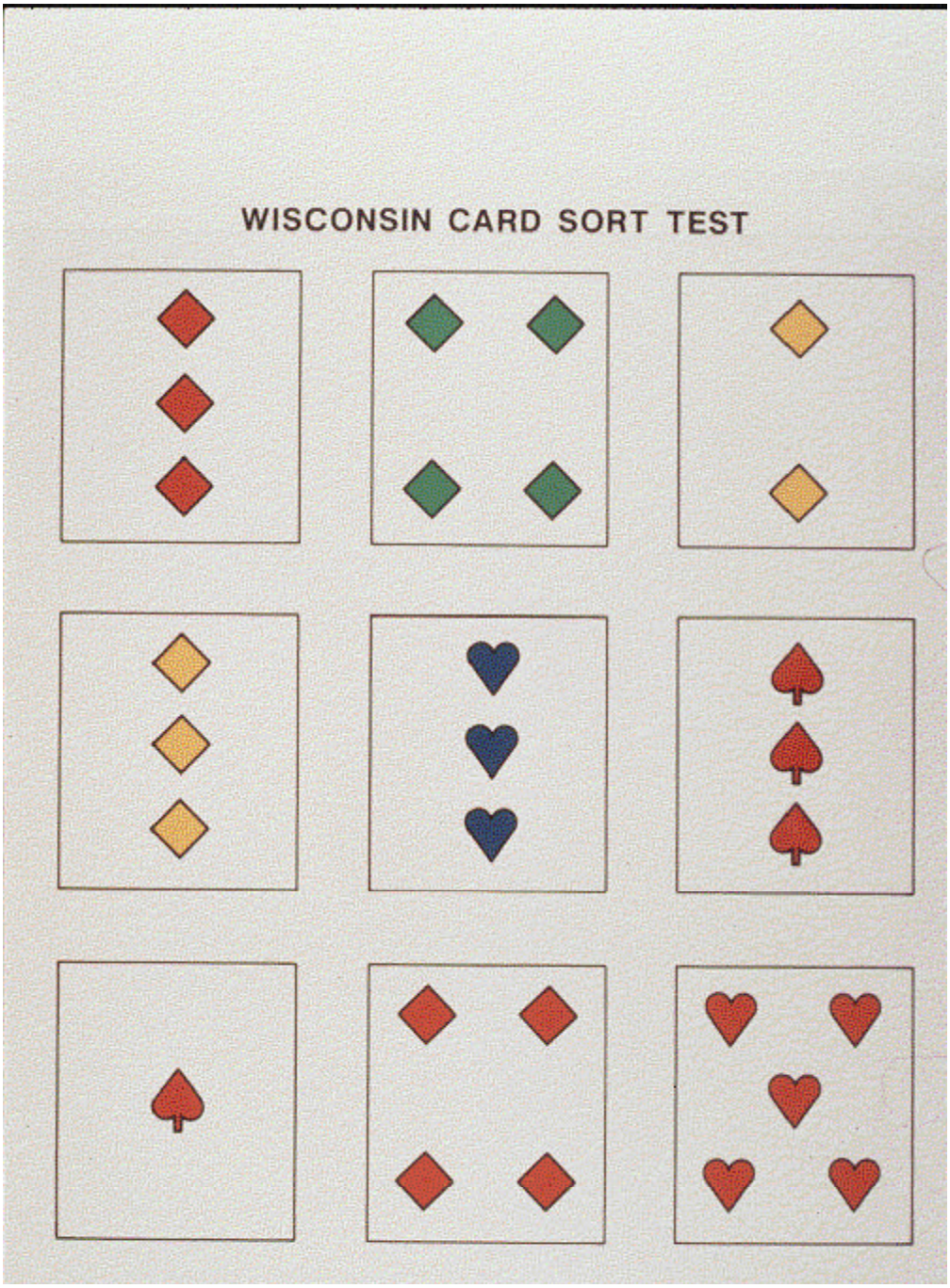
Test of Learning and Memory

- Common
  - active avoidance
  - passive avoidance
- Better
  - schedule-controlled operant behavior
  - olfactory discrimination
  - delayed alternation
  - radial arm maze -- reference and working memory

Common tests used to assess learning and memory are active and passive avoidance, which are extremely crude tests. They require the animal to either move to a lighted compartment to avoid a shock, or stay in a lighted compartment to avoid a shock. These tests take advantage of the fact that rats would rather be in a dark compartment than a light compartment. This is really kind of the same as assessing I.Q. by asking you your name. If you can't do it, that's important information. But if you can do it, it doesn't say much about your cognitive ability. Maybe I'm overstating the case slightly, but I just want you to understand that active and passive avoidance provide a very crude assessments of central nervous system integration.

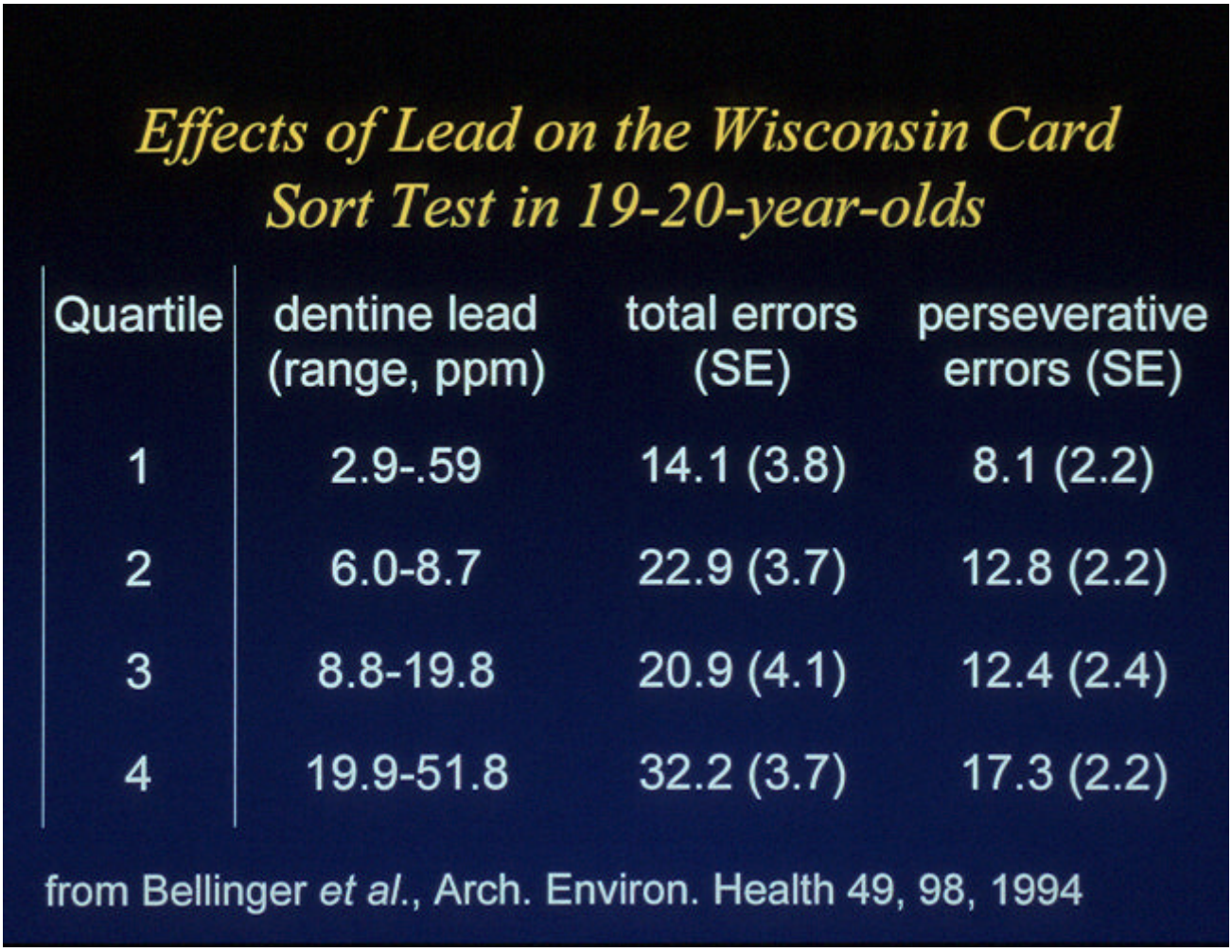
Another task suggested in the US EPA guidelines is olfactory discrimination. Olfactory discrimination is rarely used, which is unfortunate, because rats are not very visual animals. They get a large part of what they know about the world

through their olfactory system. Half of the primate brain is devoted to visual processing and integration, which is not true of the rodent. Rodents learn tasks in which the cues are olfactory readily, and this type of task should be more widely utilized.



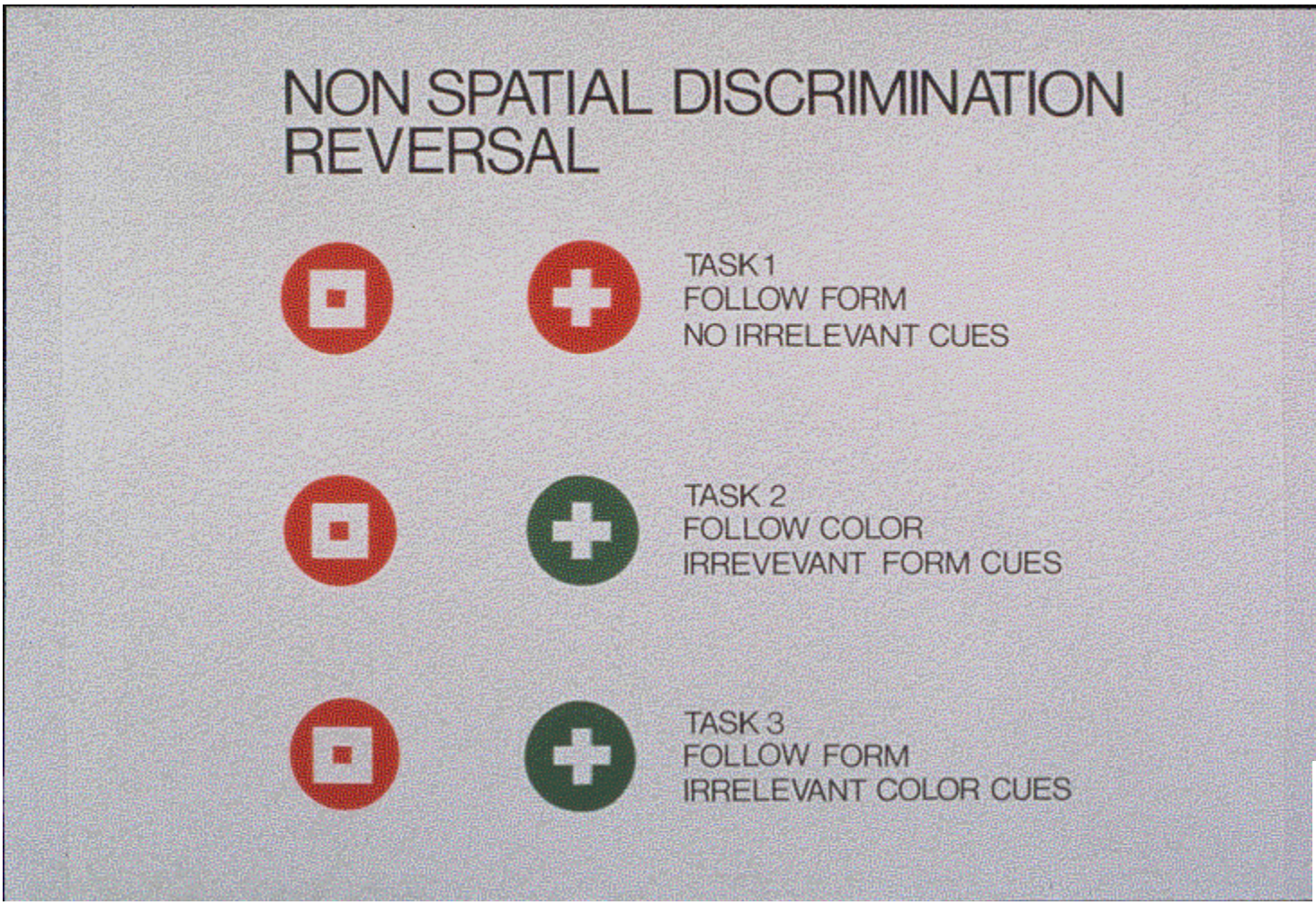
I would like to present a task that is used in humans to draw a parallel between a test that's used clinically in humans and one suitable for animals. In the Wisconsin Card Sort Test, the subject is presented a series of playing cards, and is asked

to choose a card from a series of cards that match the sample display. However, the person is not told whether they have to match based on color, number, or suit. They have to figure it out by getting choices right or wrong. After the subject figures out the rule, the rule is changed. The person is not told that the rule has changed; he or she must figure it out by having choices labeled right or wrong. It really is a very good way of looking at certain central nervous system processes that, in humans at least, utilize prefrontal cortical circuits.



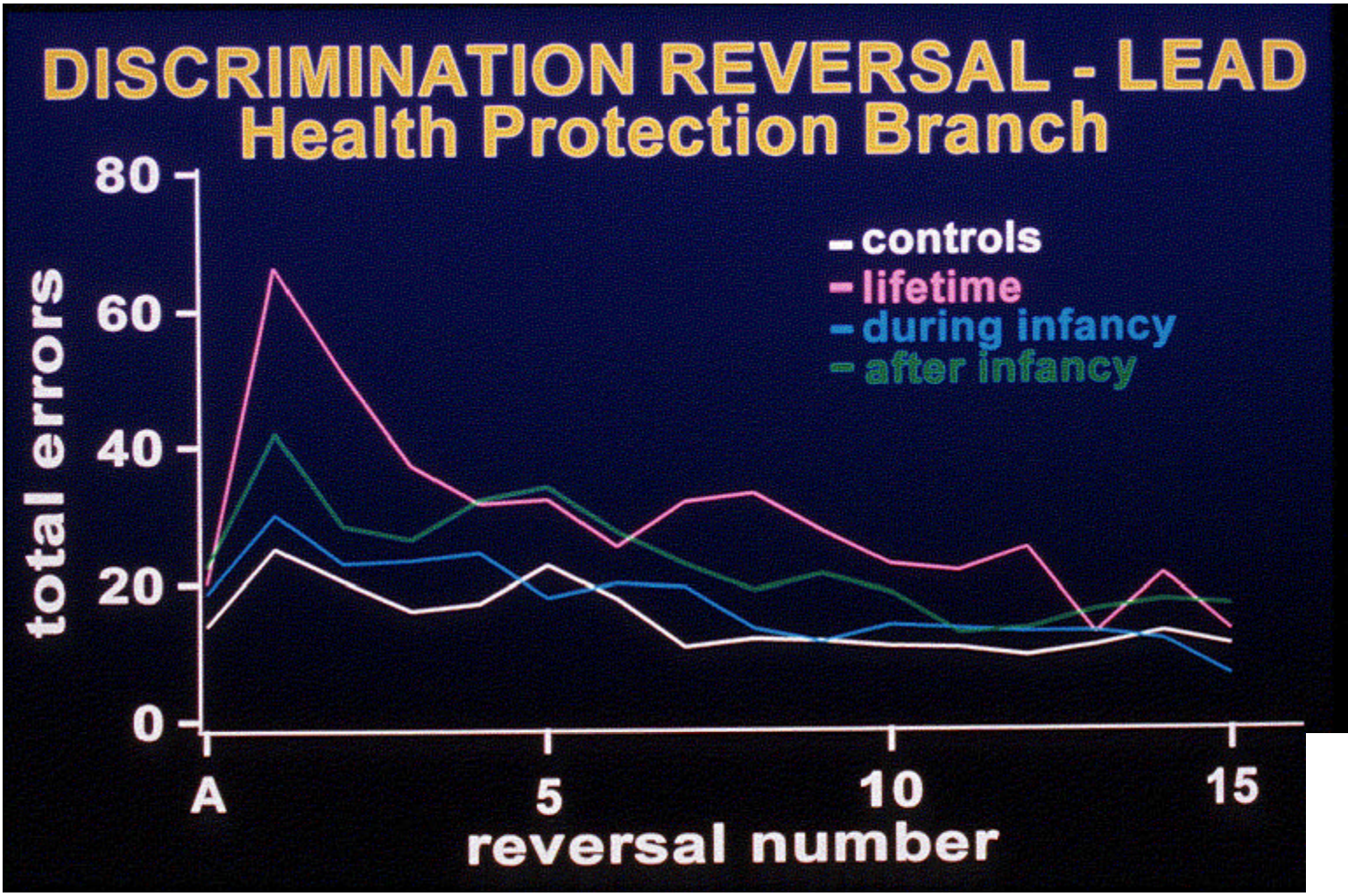
This slide shows the results on the Wisconsin Card Sort Test (WCST) in 19- and 20-year-olds associated with concurrent blood lead levels. There is an increase in total errors associated with an increase in lead levels. Bellinger *et al*. also found this in their prospective study. They found an increase in errors in 10-year-olds, associated with

concurrent body burdens of lead, and not body burdens of lead at two years of age where they found the greatest concordance with I.Q., for example.

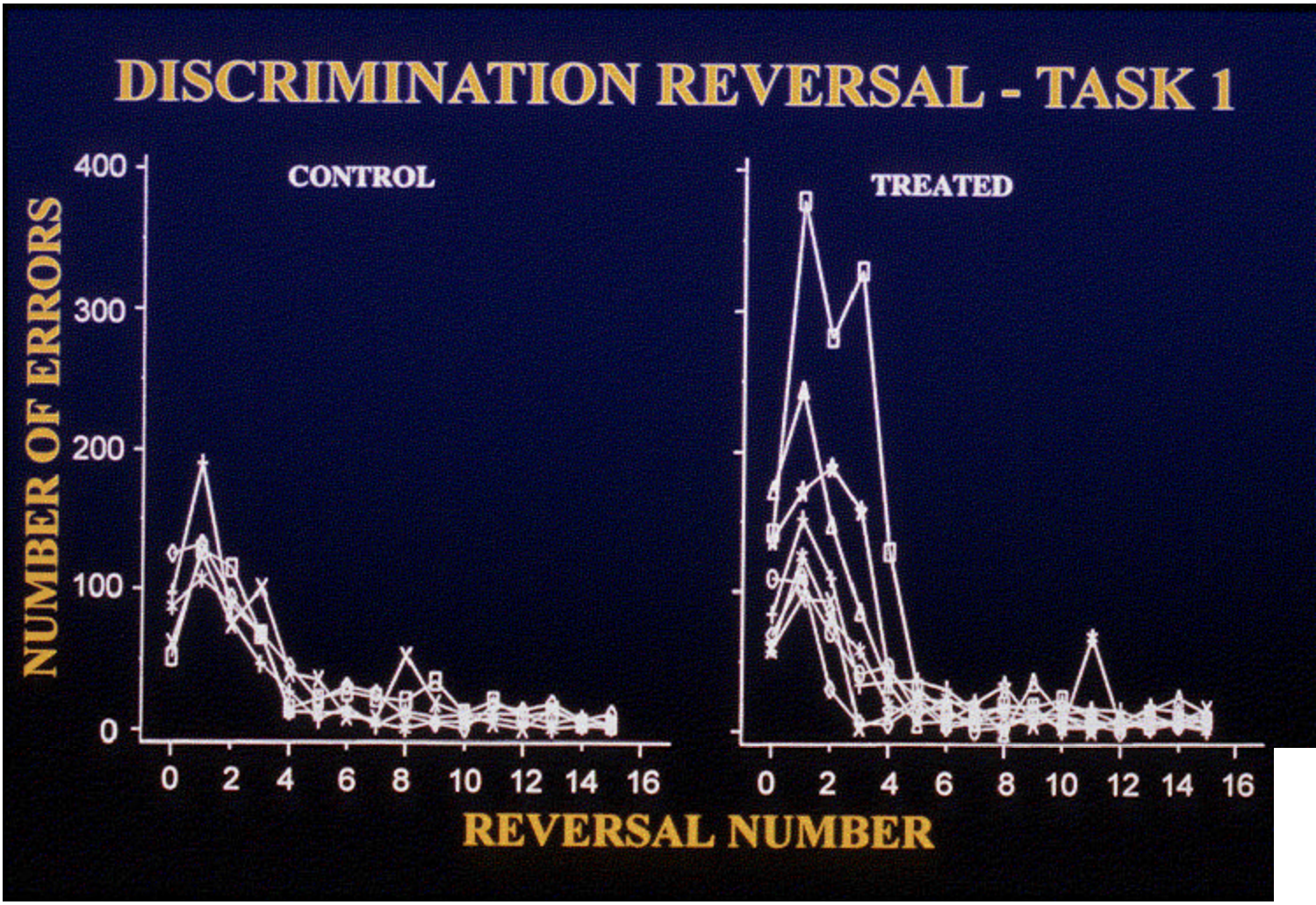


An animal version of the Wisconsin Card Sort Test is called a discrimination reversal task. A version of this can be performed by rats as well as by other animals. As in the WCST, the subject is required to learn the rules of the task by trial and error. The subject may have to learn to always respond on the cross, whether it is on the left or the right, and as soon as that is learned the correct stimulus is switched to the square instead. That is called a reversal. This test can be made even more analogous to the Wisconsin Card Sort Test. For example, the relevant stimulus dimension may change from “attend to the color” to “attend to the form”. The stimuli remains the same but suddenly the requirement changes from pay attention to the cross and the square to

pay attention to the colors. This type of task obviously assesses functions that simply learning a discrimination task will not. It turns out that very often it is much more sensitive to exposure to a neurotoxicant.

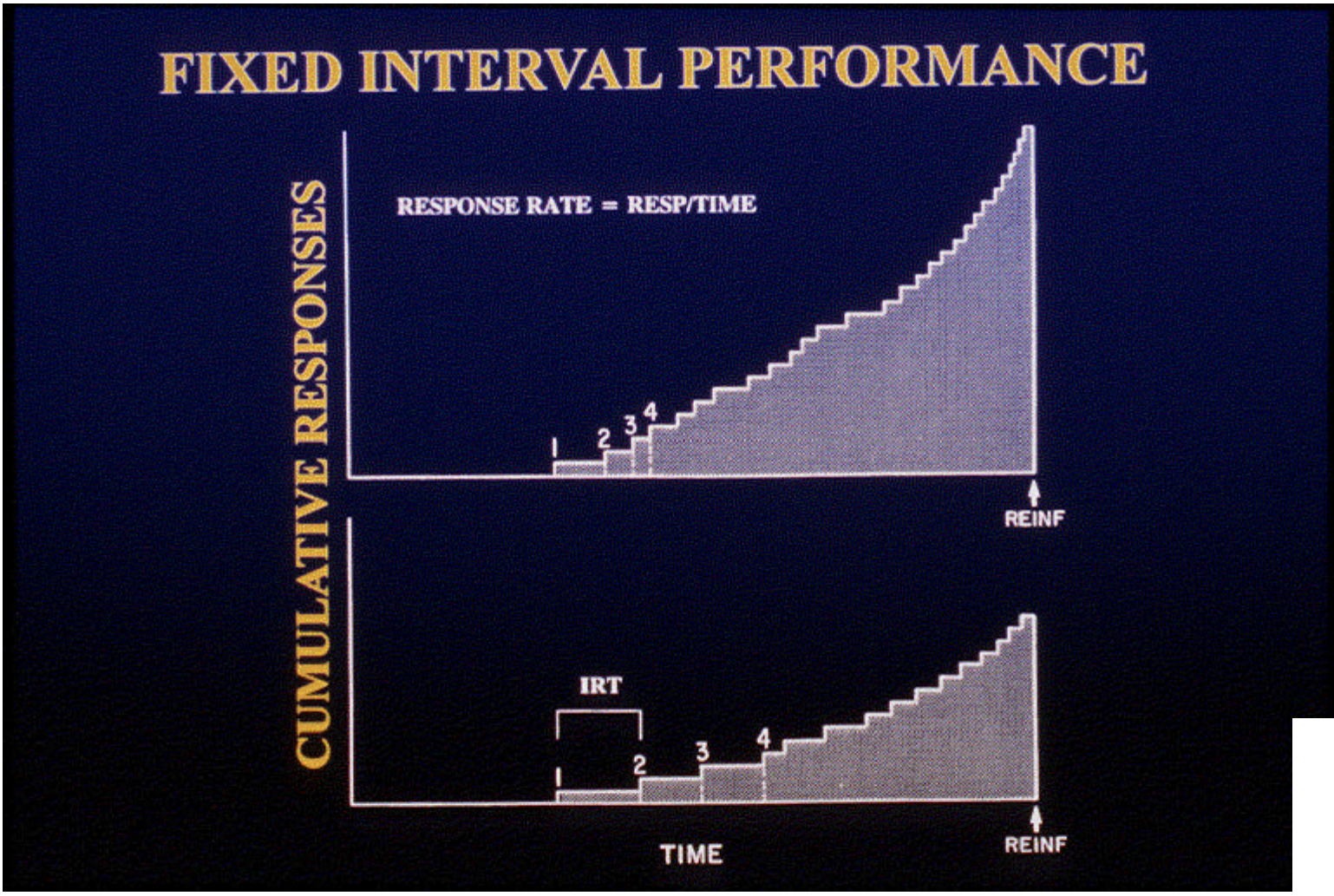


Here are data from our laboratory on the effects of lead on discrimination reversal in monkeys. A is the initial acquisition of the visual discrimination, and then 15 reversals were run. The lead-exposed animals are seriously impaired on the first reversal as measured by the total number of errors.



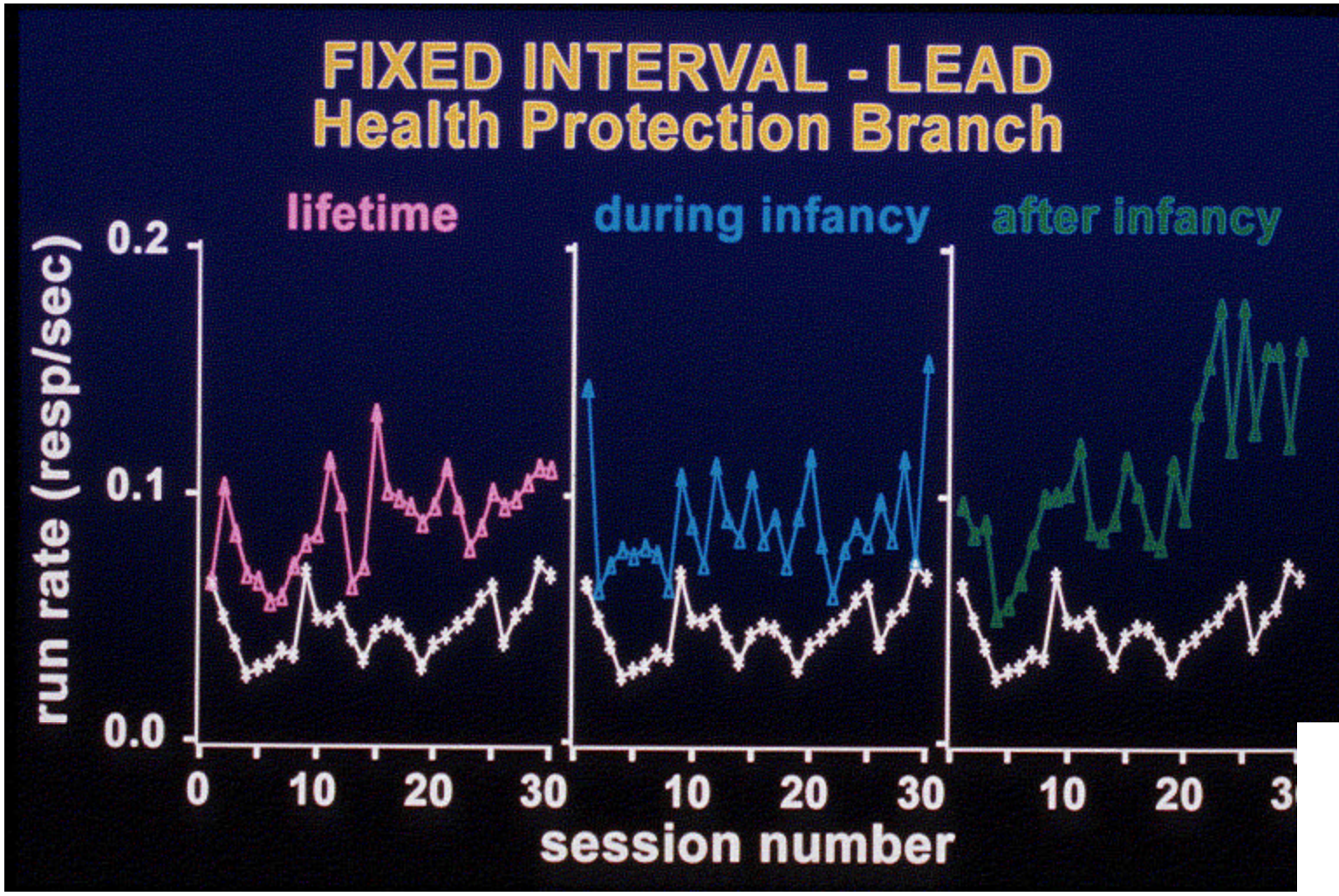
In another experiment in our laboratory, in which groups of monkeys were exposed during different periods of development, this increase in errors was observed in all treated groups.

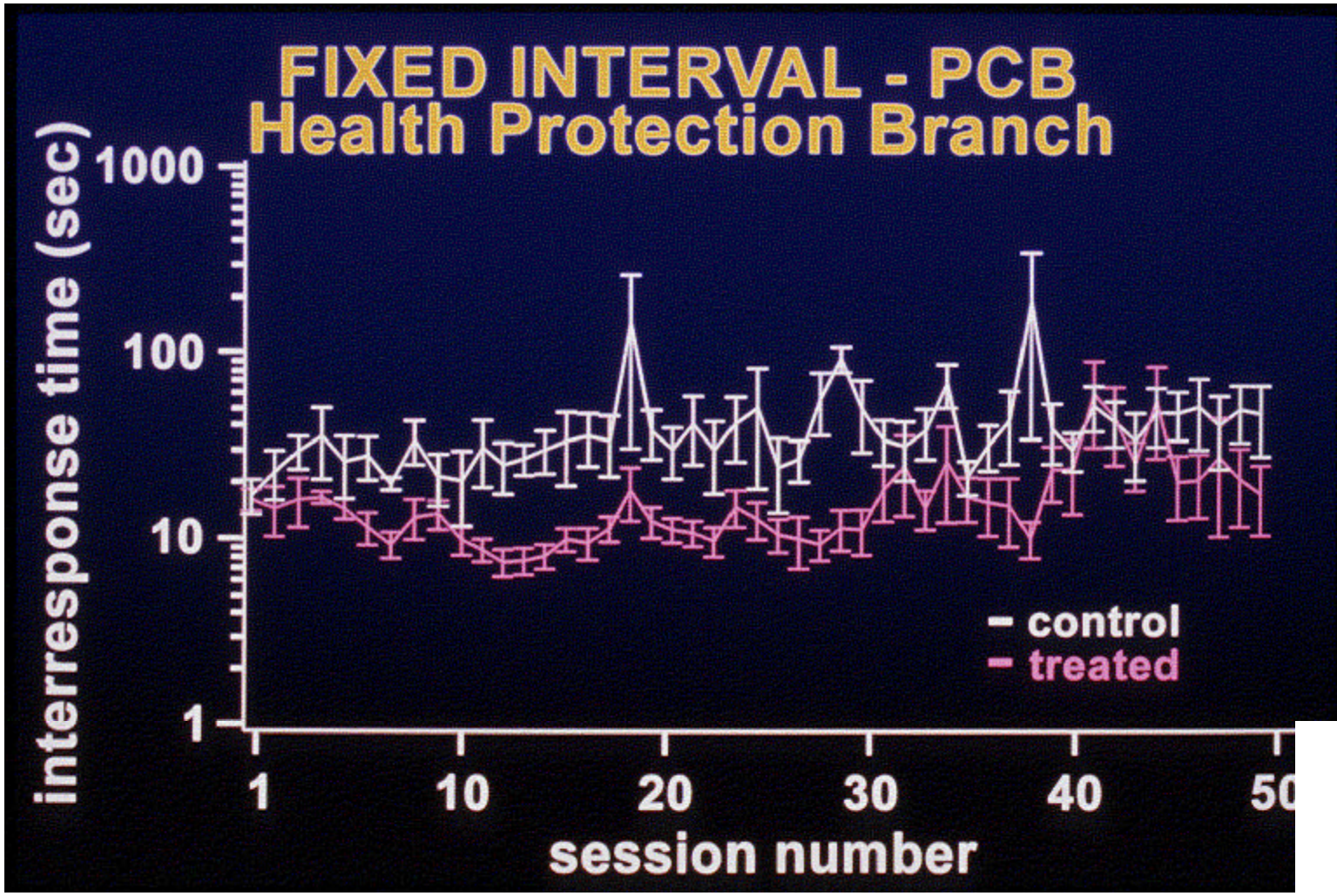
The next slide shows data from rats from a study by Susan Schantz .She observed a first reversal effect in males exposed to Aroclor-1254, but not in females. It is not unusual to find differential effects in males and females in behavioral experiments in rodents exposed to PCBs.



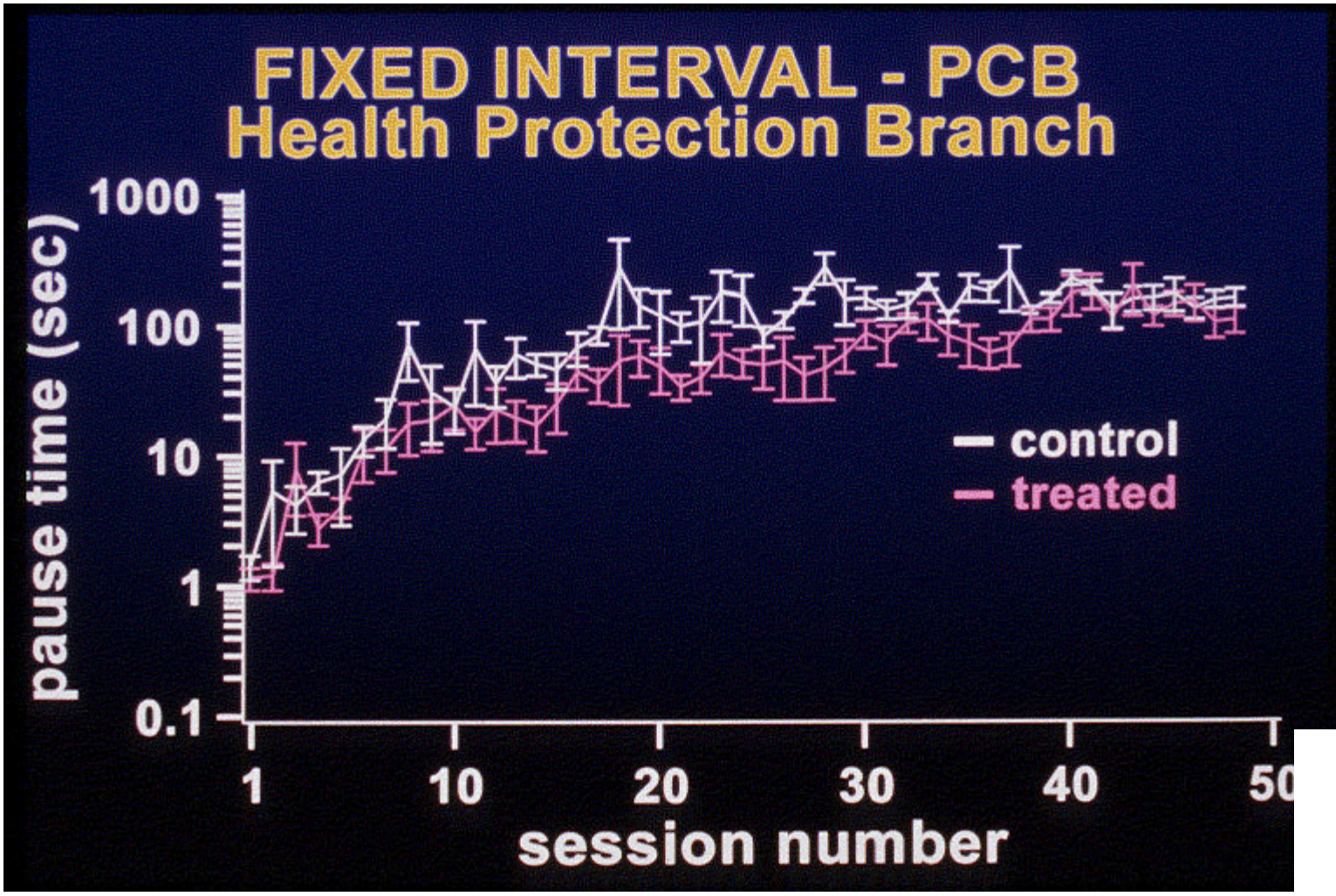
This slide depicts schematics of fixed-interval performance, which is specifically mentioned in the US EPA guideline on schedule-controlled behavior. Fixed-interval (FI) performance requires the animal to make one response and one response only at the end of a predetermined interval in order to be reinforced. What subjects actually do is pause at the beginning of the interval and then respond at a gradually accelerating rate which terminates in reinforcement. A number of endpoints can be ascertained on FI performance, including response rate, which is responses per unit time, or the inverse of that, the distribution of time between responses (the inter-response time, or IRT). Acquisition (learning) can also be assessed, as well as the pattern of responding across the interval.

The next slide depicts FI performance in different species and under different conditions: e.g., in the pigeon working for water or food, rat pressing a lever, chimpanzee, rats running in a wheel, cats, and it is apparent that in every case a very nice scalloped pattern of responses is present, which is typical of FI performance. Humans also emit this pattern of fixed-interval performance. The next slide shows run rate on the FI schedule in monkeys in our laboratory exposed to lead. All groups exposed to lead developmentally exhibited an increased rate of response compared to controls. This is not incorrect performance, but it is certainly inefficient performance because treated monkeys are making many more responses for each reinforcer. The same effect is observed in rats as demonstrated by Deborah Cory-Slechta. In fact when you ask lead-exposed animals to inhibit their performance by doing another kind of task, you find that they can't do it.

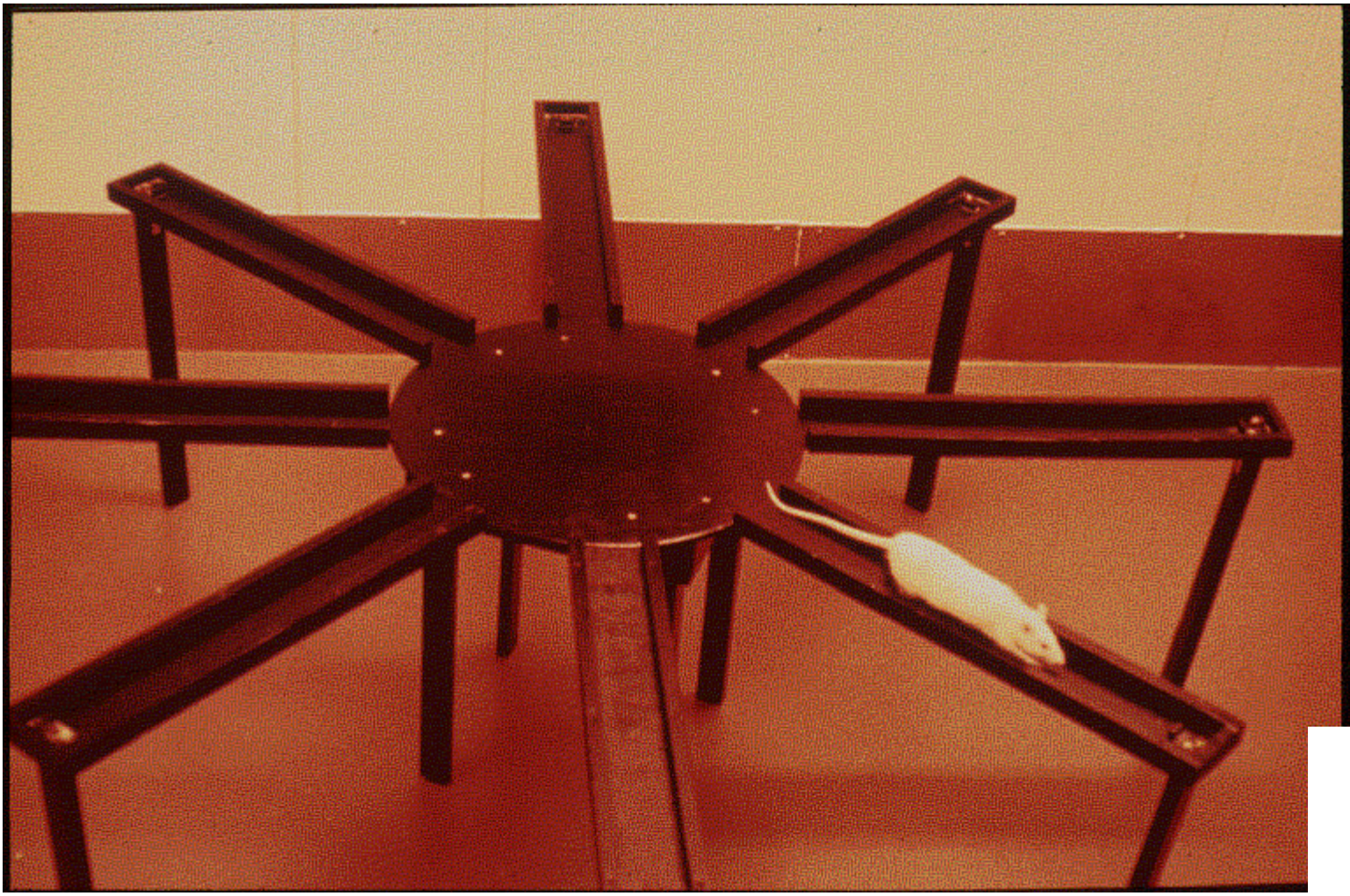

FIXED INTERVAL - LEAD
Health Protection Branch
lifetime
during infancy
after infancy
run rate (resp/sec)
0.2
0.1
0.0
0
10
20
30
10
20
30
10
20
session number



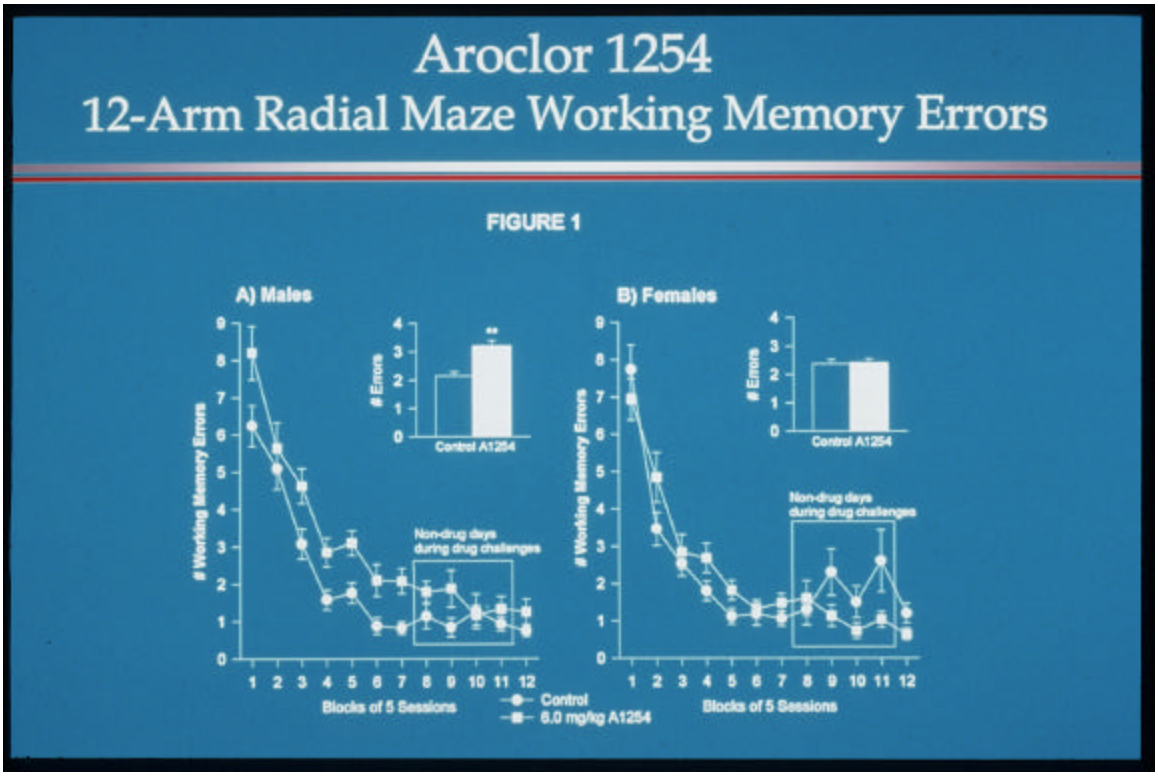
The next slide depicts inter-response time (reciprocal of response rate) for performance on a 6-minute FI schedule. Monkeys were exposed only postnatally to a PCB mixture and tested during adulthood, long after exposure had ceased. The treated monkeys have a shorter time between responses; in other words, they, like lead-treated animals, are responding at a higher response rate than are the control animals.



This slide depicts the pause time for the monkeys just described exposed to PCBs across 50 sessions (or 50 days of testing). The pause time starts out very short at the beginning of the experiment, about one second. By about session 20 or so, the control animals are pausing about a hundred seconds before their first response, which is then stable for the remainder of the experiment. The treated animals, in contrast, take about another 20 sessions to reach the same value.

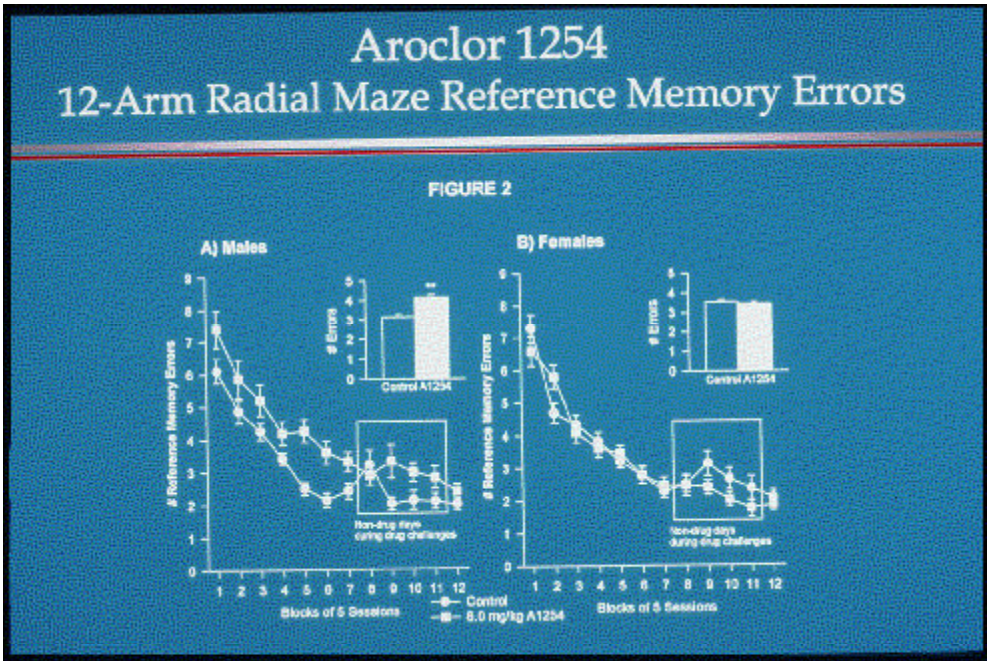


This is a picture provided by Susan Schantz showing a radial arm maze. The radial arm maze is used quite often in rodent work. This picture is an eight-arm radial arm maze, and you can see a rat going down one arm. Some arms may be baited with treats on every test day, while other arms may never be baited. This allows assessment of different memory processes. Entering alleys that are never baited would be considered errors in reference memory. Reference memory may be considered roughly analogous to remembering your phone number. Working memory, on the other hand, may be considered to be more immediate memory: i.e., the subject has to remember from moment to moment whether he has been down a particular alley today, like remembering where you left your keys.

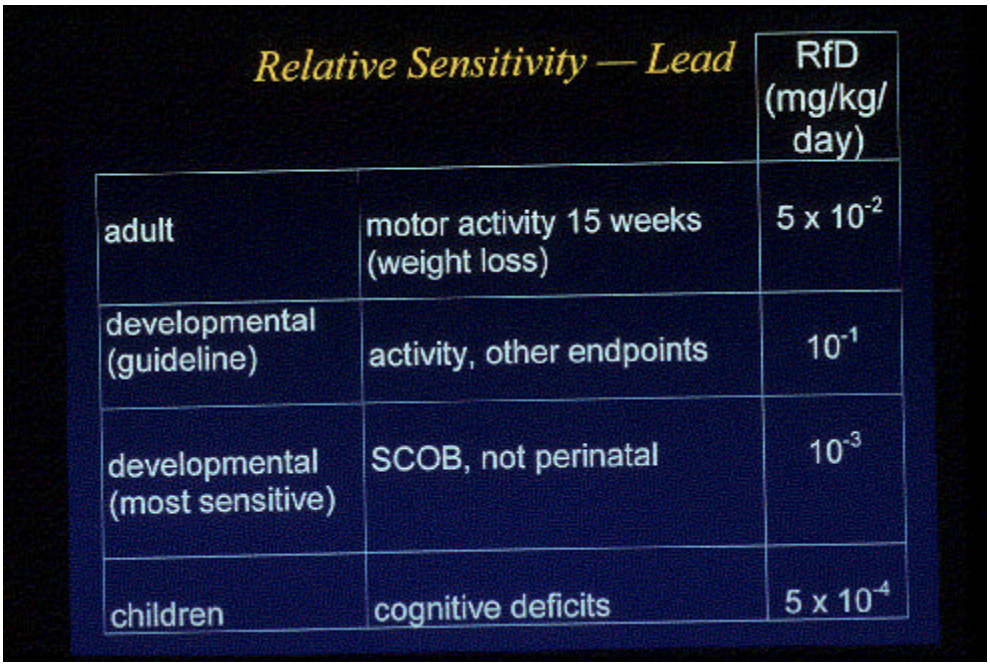


Roegge C. et al., Tox. Sci. 57, 121-130, 2000

These types of memory may be differentially affected by toxicants.These are data from Sue Schantz's lab with Aroclor-1254 using a 12-arm radial arm maze. She found an increase in working memory errors in males and not females.



This slide shows data for reference memory errors from the same study. Again effects were observed in males and not in females. But there may in some cases be a differential effect in which, for example, working memory is affected, but reference not memory.



Relative Sensitivity — Lead

		RfD (mg/kg/day)
adult	motor activity 15 weeks (weight loss)	5×10^{-2}
developmental (guideline)	activity, other endpoints	10^{-1}
developmental (most sensitive)	SCOB, not perinatal	10^{-3}
children	cognitive deficits	5×10^{-4}

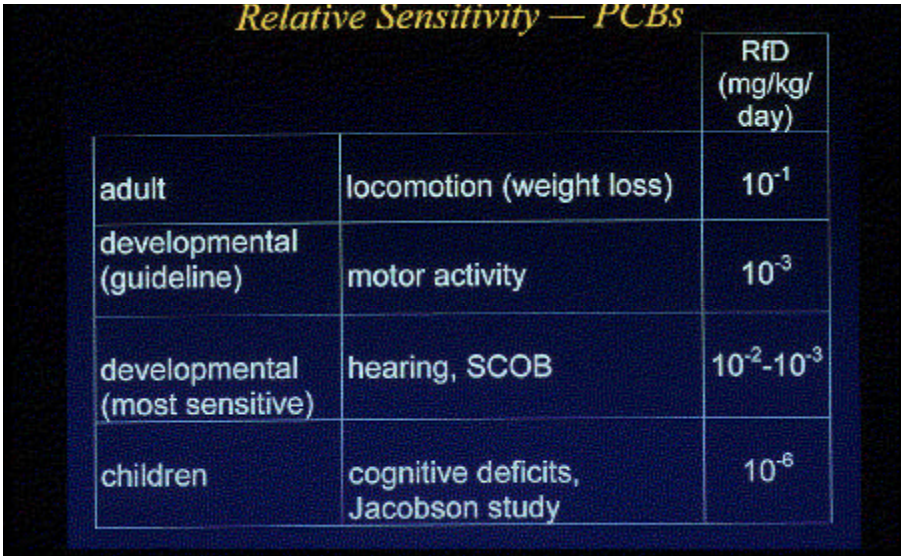
Slide 2

Here are a series of RfDs that I calculated using lead as an example. I have based the calculated RfDs for animals based on data from rats, using the study that yielded the most sensitive RfD in each category. For adult rats, the most sensitive study found changes in motor activity after 15 weeks of dosing. Decreased motor activity was associated with significant weight loss, and so probably does not represent a neurotoxic effect. The RfD is 5_10^{-2} mg/kg/day. (I added the extra factor of 10 for the interspecies extrapolation between animal studies, but I didn't add any additional ones. I didn't add, for example, for this adult study a less-than-lifetime exposure; so keep that in mind as I go through this.) For developmental studies, a study reporting changes in activity, as well as other endpoints, results in an RfD of about 10^{-1} mg/kg/day. For developmental studies,

the most sensitive studies in the literature are research by Deborah Cory-Slechta using schedule-controlled operant behavior, specifically fixed-interval performance. Exposure was not perinatal; dosing began post-weaning. These studies yield an RfD of about 10^{-3} mg/kg. For derivation of an RfD in children, I really tried to be the least conservative I could be. For the threshold for effect I used 10 micrograms per deciliter, which is probably too high. Then I used published estimates for the amount of lead in various foods and intake of various foods by children, and derived a RfD of 5_10^{-4} mg/kg/day. According to this example, a comparison reveals that the estimate from adult rodents is low by about two orders of magnitude. I may point out that a 15-week activity study would probably not have been performed if it were not already known that lead is neurotoxic based on human poisoning. If data from typical endpoints used in developmental studies are considered, the inconsistency is about three orders of magnitude.

This conclusion is in contrast to what I said at the beginning of the talk, which is if you look at body burden the congruence between humans and animals is actually very good. However, if dose is used the correspondence is not protective of human health. Perhaps one of the things that needs to be done is a requirement for kinetic and mechanistic data from animal studies.

One of the reasons I presented in detail the kinds of tests that are available for use in animals is that they can provide data at low body burdens that are comparable to effects found in children.



If the same kind of analysis is performed for PCBs, again the most sensitive study in adults is a 15-week study in rats by the same investigators as the lead study. Decreased activity was observed in the presence of profound weight loss, and so probably does not represent a neurotoxic effect. The RfD based on this study would be 10^{-1} mg/kg/day. The most sensitive study consistent with the developmental guideline in motor activity, which yielded an RfD of 10^{-3} mg/kg/day. Other developmental effects, hearing deficits and effects on schedule-controlled operant behavior, yield an RfD in about the same range as motor activity. These calculations are based on commercial mixtures. Considering data from children, cognitive deficits identified in the Jacobson study yields an RfD of about 10^{-6} mg/kg/day. Ongoing studies may result in an RfD lower than that. If using the neurotoxicty guidelines, based on dose, in developing organisms, the difference between human and rodent data is three orders of magnitude. If adult rodent data are used, which probably do not represent neurotoxicity, the difference is five orders of magnitude.

I have used lead and PCBs as an example because these are two agents for which there is an extensive literature in humans and animals. Probably the other chemical for which this is true is methylmercury, which would yield similar results as lead and PCBs. I would suggest that this analysis is relevant to the issue of the FQPA requirement to include an extra factor of 10 to protect children's health. Based on these

examples, an extra factor of 10 based on external dose would not be sufficiently protective even based on data from developing organisms. We need a new approach to this issue.

And like Dr. Faustman, I would really like us to get to the point where we're not doing these experiments in humans but right now we are. And this is what the data are telling us, we cannot do RfDs based on dose, it just isn't nearly protective enough.

Thank you. (Applause.)

Questions and Answers (Panel Discussion)

DR. GOLUB: Thank you, Debbie, and I think we can just sort of stay where we are and take questions from the audience. I think this is our last session before lunch. Any questions for Dr. Rice, or following that, Dr. Rice or Dr. Barone?

DR. BARONE: I have a question, maybe clarification. You say dose, are you talking primarily about administered dose and not necessarily about body burden or target tissue dose?

DR. RICE: Well, I mean if you do body burden the congruence is really good. DR. BARONE: I just wanted to underscore that again.

DR. RICE: Yeah, exactly. But if you look at administered dose it's awful. People talk about what more sensitive tests can we use and really that's not the problem. The animals are good models if you use body burden, they really are.

DR. ROGAN: I'm Walter Rogan from NIH.

Hugh Tilson and Joe Jacobson and I did an exercise not unlike this one a few years ago, and when we have talked to our regulatory colleagues about it one

argument we get about this approach is that everybody's already exposed above this number, therefore it can't be right.

So I don't really know where this goes in this conference, but if you actually start using the human data from PCBs and from lead, and probably from methyl mercury and some other things, then you're going to be stuck with the fact that if Joe Jacobson and I, and the people in New York and the people in Holland, see effects from PCBs, that means that people are above where they're supposed to be, and if we see it in the top 10%, then that means that almost everybody's above, because you're supposed to be off by 10.

I'm just wondering whether the feds or the state are kind of prepared to do a better job with that in the next 10 years than they've done.

DR. RICE: I'm kind of wondering that too.

DR. ROGAN: I don't know if that's a question or a comment, but it's going to happen, you're going to end up -- the more sophisticated you start approaching these things, and as you get into non-cancer end points, and as you start dealing with things from kids you're going to find yourself in a situation where your number is below the exposure of substantial fractions of your extant population, and you're going to have to be prepared for the repercussions of that, because it's not going to be 800, it's going to be right in the middle.

DR. BARONE: Well, I think you have an extremely important point, and it's an issue that we hear all the time in sort of the Office of Research and Development, you know, how much more work do we need on lead, mercury and PCBs when we know these things are bad actors.

But in the context of chemicals, high production-volume chemicals and also pesticides, herbicides, fungicides, and cholinesterase inhibitors, insecticides, I think

there's a lot of important lessons we've learned over the last 25 years about the exposure response relationships. And I think we are designing -- we have a program and many in academia and the children health centers have programs focused on mode of action and mechanism of action, and try to deal with aggregate and cumulative exposure. I think that's going to be a real challenge for us.

DR. ROGAN: From the point of view of being in the primary production of data business, though, it's disappointing when there's not going to be another substance, we hope, that's going to permit the collection of a body of data such as there is for lead and PCBs and methyl mercury, because you have the animals and you have the people, and you have positive data from both. So it's a little dismaying, from my end, to hear the regulatory agencies talk about more sophisticated protocols when we have all the data already and nobody wants to go dredge the Hudson River.

So saying, you know, if we only had a better test battery for identifying things -- but when you've identified them and you've got data for 15 years -- and, see, there's always this argument about, well, you know they're not perfect, but they're better than we're ever going to have on any new substance, and yet we still can't get there, so.

DR. RICE: Yeah, I can't imagine that we could have any more compelling data than we already have.

DR. PORTIER: Chris Portier from NIEHS. Thanks for a fascinating, exciting talk.

I think the lead and the PCBs and the methyl mercury issues are rather unique in terms of body burden in the sense that the body burden doesn't change dramatically as a function of short periods of time, and so it's easy to look at it. I think as we look at other developmental toxicants it's going to be a lot more difficult to make that

comparison on body burden basis, and I think we're going to have to think a little bit more about the design of studies to be able to address that question.

But I think you point to a very interesting phenomenon in the sense that all of this information that you're looking at here, or much of it, in terms of body burdens and effects as a function of body burden could have been done 10 years ago if we'd have carefully analyzed some of our data just on background levels and classifying people into groups, and I think that's a lesson to be learned here.

The last point I had was on the FQPA issue. One thing which is very interesting in the context of looking at RFDs for the future is that the comparison you made here is pretty much on the basis of LOAELs and NOAELs and observable effects and where they fall. The future is looking towards equivalent-risk units, so 1% risk, a 10% risk, finding that and basing your RFD on that.

As we go to the question of the application of a 10-X safety factor, and California's going to be facing the same issue it seems, you have to think about the situation where you have the perfect epidemiology study for children, where you in fact have carefully understood exposure, carefully measured response, a perfect dose response pattern, a model that matches the data, clear understanding in the mechanism, and you're going to set your point of departure at 10% or 5% risk. You would clearly still need some sort of factor to move you out of that range, and I think that's where we're failing to think about where our safety factors really, rather than uncertainty factors, have to fall.

DR. RICE: Yeah, I agree with you completely. As we go to benchmark dose and more sophisticated approaches, I don't think it would change the slides that I put up there very much. But you're absolutely right, those are the kinds of things we're going to have to think about -- okay, we have a 10% risk and now what do we do.

DR. GOLDMAN: You can make a lot of comments based on that. I've heard versions of your talk before and it's just wonderful the way you're able to put the data together from a lot of different kinds of studies. I think it's something that we too infrequently see, and especially the melding together of the human data with the animal tox data to get a clearer picture of some of the flaws in the way that the reasoning has been used for many, many years in toxicology.

I guess it's really more of a comment and a suggestion to California that maybe in this new effort that you're undertaking, a way to approach this might be to look at areas like this where perhaps EPA is beginning to recognize the issue but hasn't quite moved forward into the issue as an area of opportunity for being able to move things forward in terms of public health protection, and particularly I'm talking about addressing body burden and not just intake levels, and doing risk assessment.

The other thing that occurs is how this points to how really important it is to understand what the actual exposures are in the population. I know that the Centers for Disease Control, Center for Environmental Health under Dick Jackson, that they are going to be very much increasing the amount of information that they'll be pulling out of the National Health and Nutrition Examination Survey on exposure levels to toxic chemicals, and particularly they're going to attempt -- and I don't know if it's going to work because of how the survey is designed -- they're going to attempt to, on an annual basis, give the country some kind of a report card about what those exposure levels are.

I think that's absolutely critical for most of the compounds that we're concerned about. We don't actually have information about population exposures. But unfortunately NHANES is not going to tell us much about exposures to kids, and it's not going to tell us much about what's going on prenatally.

I guess I want to suggest that's another place where California might look at being able to provide a contribution that the federal government, in the short term, isn't going to do. That would be either looking at prenatal levels, breast milk levels, or other indicators, and where I know that there are a lot of opportunities because of the kind of surveillance that California does, and the kind of material that's available for doing that kind of analysis. DR. MARTY: Actually, just a comment too. I think that, you know, the two examples of PCB and lead are examples where we really haven't taken into account pharmacokinetics at all. When we set exposure levels for chemicals that have very long half-lives in the body, and where a short exposure is really a chronic exposure, it's not really an acute exposure because this stuff hangs around forever. It's very interesting to see that the body burden information between animals and humans, there's a congruence there, but certainly not in the dose, and I think that's probably at least one of the reasons.

And a risk assessment paradigm sort of breaks down when you look at chemicals that have a very long half-life, and we don't take into consideration these pharmacokinetic issues. This applies not just to chemical-specific risk assessment, but also to site-specific risk assessment. There have been many, many times we've been approached by a local air pollution control district; they have a project that they want to permit but it's going to expose people for X number of months or a couple years to diesel exhaust or, you know, whatever the chemical, and they want to know if they can use our little black box risk assessment method to evaluate these shorter-term exposures. We never have an answer; we just don't have an answer for that.

Also another comment. We too are running into this issue of, well, we're being exposed at those levels when we're trying to set reference exposure levels for airborne toxicants or public health goals for water, we keep running into this issue. But we're already exposed at that level, so your number must be wrong and your uncertainty factors are too big. I think we're just going to keep running into that more and more. So, there's a lot of work that needs to be done to help us figure out these particular issues and how to deal with them.